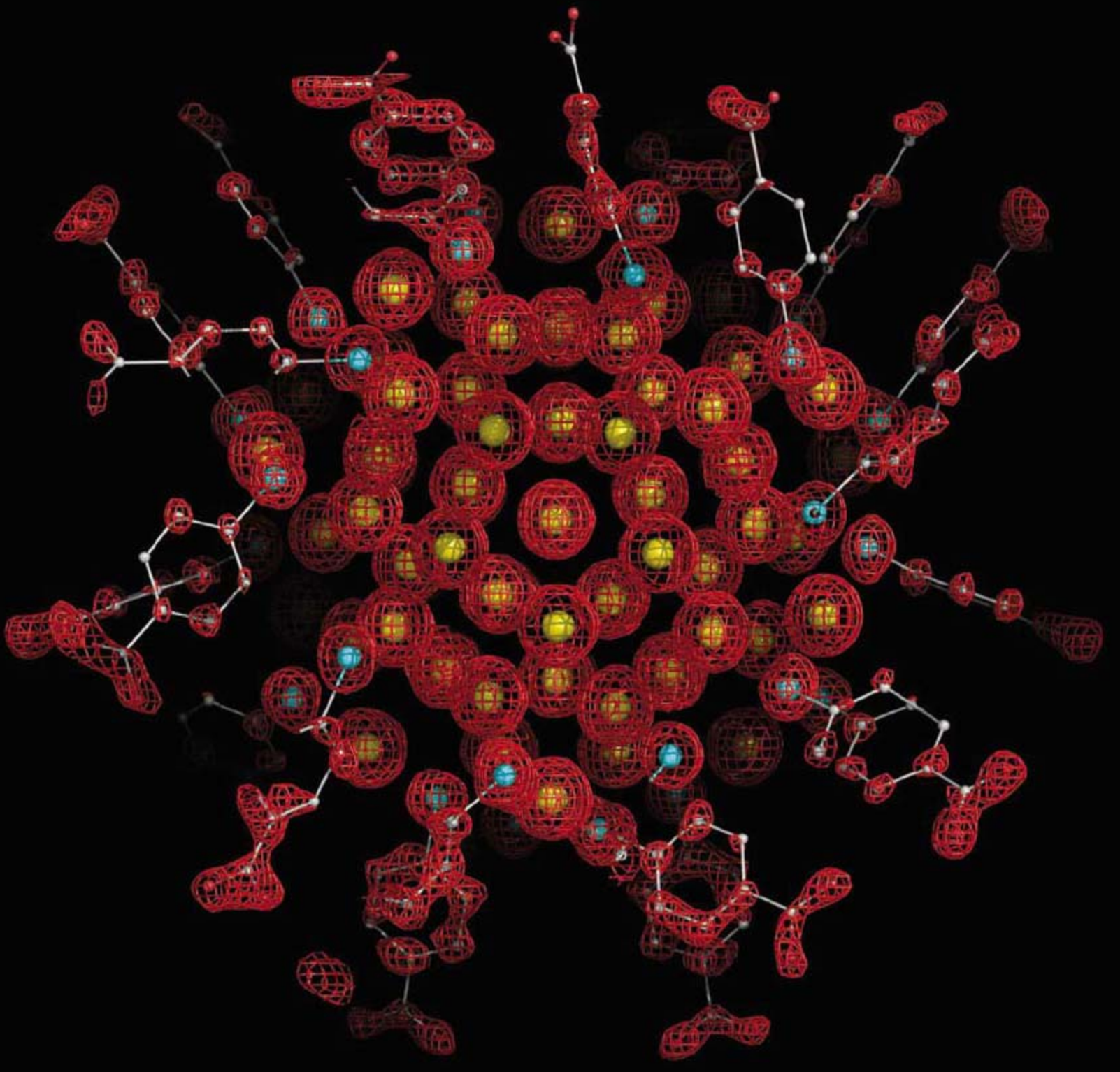
19 October 2007 | $10

Science



AAAS

Volume 318, Issue 5849

COVER

Structure of a gold nanoparticle in which the central atoms are packed in a decahedron, surrounded by additional layers of gold atoms in unanticipated geometries. Gold atoms, gold; sulfur atoms, blue; carbon atoms, white; oxygen atoms, red; the superimposed red mesh depicts the electron-density distribution determined by x-ray crystallography. See page 430.

Image: Pablo D. Jadzinsky and Guillermo Calero

DEPARTMENTS

353 *Science* Online
355 This Week in *Science*
361 Editors' Choice
364 Contact *Science*
369 Random Samples
371 Newsmakers
479 *Science* Careers

EDITORIAL

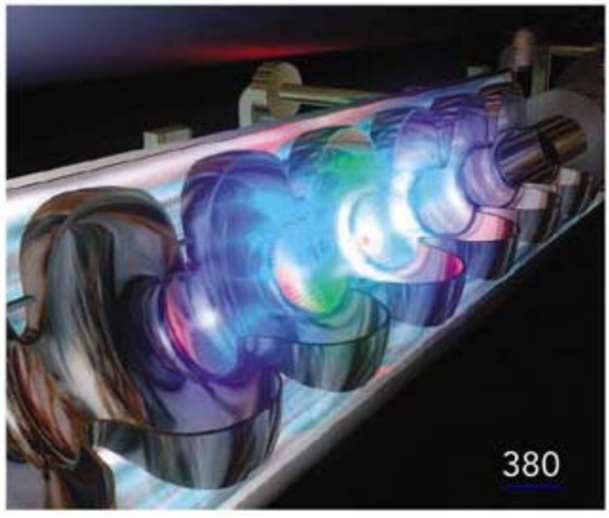
359 Feeding a Hungry World
by Norman Borlaug

NEWS OF THE WEEK

Nobel Peace Prize Won by Host of Scientists and One Crusader 372
Chemistry Laureate Pioneered New School of Thought 373
Three Economists Lauded for Theory That Helps the Invisible Hand 375
SCIENCESCOPE 375
Natural Selection, Not Chance, Paints the Desert Landscape 376
Coastal Artifacts Suggest Early Beginnings for Modern Behavior 377
Space Sighting Suggests Stardust Doesn't Have to Come From Stars 379
U.K. Spells Out Boost in Medical Research 379

NEWS FOCUS

Location, Location, Location 380
Fresh Evidence Points to an Old Suspect: Calcium 384
Dirty Science: Soil Forensics Digs Into New Techniques 386
In Search of the World's Most Ancient Mariners 388



LETTERS

Of Aging Mice and Men *R. Miller* 390
Response *H. Liu and T. Finkel*
Replicating Genome-Wide Association Studies
M. H. Kuniholm Response *D. B. Goldstein*
A Measure of Respect for Translational Research
A. Sharma
CORRECTIONS AND CLARIFICATIONS 393

BOOKS *ET AL.*

A Farewell to Alms A Brief Economic History of the World *G. Clark, reviewed by S. Bowles* 394
Are We Ready? Public Health Since 9/11 396
D. Rosner and G. Markowitz, reviewed by P. S. Keim

396

POLICY FORUM

The Science and Business of Genetic Ancestry Testing 399
D. A. Bolnick et al.

PERSPECTIVES

Sampling the Sun 401
K. Marti
>> Report p. 433
A New Twist for Mercury 402
C. Lamborg
>> Research Article p. 417
Unlocking Cell Fate 403
A. G. Rivenbark and B. D. Strahl
>> Reports pp. 444 and 447
Simmering, Not Boiling 404
C. R. Kaiser
Why Genomes in Pieces? 405
L. F. Landweber
>> Brevia p. 415; Report p. 450
Nano-Golden Order 407
R. L. Whetten and R. C. Price
>> Report p. 430

CONTENTS continued >>

Science

SCIENCE EXPRESS

www.sciencexpress.org

CELL BIOLOGY

A Bifunctional Bacterial Protein Links GDI Displacement to Rab1 Activation
M. P. Machner and R. R. Isberg
The bacterium that causes Legionnaires' disease recruits a host protein that regulates vesicle trafficking by mimicking host activating proteins.
10.1126/science.1149121

GENETICS

Genome-Wide Experimental Determination of Barriers to Horizontal Gene Transfer
R. Sorek et al.
Characterization of gene families that cannot be cloned in the common bacteria *Escherichia coli* suggests that increased dosage and expression of certain genes are toxic to the host.
10.1126/science.1147112

GENETICS

A β-Defensin Mutation Causes Black Coat Color in Domestic Dogs
S. I. Candille et al.
Identification of a gene causing black coat color in dogs reveals a surprising new role for a protein family previously implicated in the body's defense against microbes.
10.1126/science.1147880

TECHNICAL COMMENT ABSTRACTS

NEUROSCIENCE

Comment on "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension" 393
N. Sanai et al.
full text at www.sciencemag.org/cgi/content/full/318/5849/393b

Response to Comment on "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension"
M. A. Curtis et al.
full text at www.sciencemag.org/cgi/content/full/318/5849/393c

REVIEW

MATHEMATICS

Mathematics and Complex Systems 410
R. Foote

BREVIA

GENETICS

Mitochondrial DNA as a Genomic Jigsaw Puzzle 415
W. Marande and G. Burger
The mitochondrial genes of the unicellular eukaryote *Diplonema* are fragmented among many small chromosomes, and their coding RNAs are pieced together to form messenger RNAs.
>> Perspective p. 405; Report p. 450

RESEARCH ARTICLES

GEOCHEMISTRY

Mass-Dependent and -Independent Fractionation of Hg Isotopes by Photoreduction in Aquatic Systems 417
B. A. Bergquist and J. D. Blum
The odd isotopes of mercury are fractionated in a mass-independent manner during photoreduction, providing a tracer of mercury species and reactions through food webs. *>> Perspective p. 402*

GENETICS

Paired-End Mapping Reveals Extensive Structural Variation in the Human Genome 420
J. O. Korbel et al.
Sequencing of structure variations over segments of DNA from two individuals of different ethnic groups showed unexpected levels of diversity.

REPORTS

MATERIALS SCIENCE

Mussel-Inspired Surface Chemistry for Multifunctional Coatings 426
H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith
A wide variety of surfaces can be coated with a slightly basic dopamine solution, creating a universal adhesive that can be readily modified by secondary reactions.

CHEMISTRY

Structure of a Thiol Monolayer–Protected Gold Nanoparticle at 1.1 Å Resolution 430
P. D. Jadzinsky et al.
Particles containing 102 gold atoms bound to 44 organosulfur molecules can be grown uniformly, allowing x-ray resolution of their pure gold core and chiral structure. *>> Perspective p. 407*

PLANETARY SCIENCE

Constraints on Neon and Argon Isotopic Fractionation in Solar Wind 433
A. Meshik et al.
Neon and argon isotopic ratios remain the same throughout the solar wind in samples returned by Genesis, ruling out some solar models that predicted changes. *>> Perspective p. 401*

CONTENTS continued >>

Science

REPORTS *CONTINUED...*

CLIMATE CHANGE

Southern Hemisphere and Deep-Sea Warming Led Deglacial Atmospheric CO_2 Rise and Tropical Warming 435
L. Stott, A. Timmermann, R. Thunell
Dating of benthic versus near-surface plankton in a Pacific Ocean core shows that southern high latitudes warmed 1500 years before the tropics during the last deglaciation.

PALEOCLIMATE

Mixed-Layer Deepening During Heinrich Events: A Multi-Planktonic Foraminiferal $\delta^{18}O$ Approach 439
H. Rashid and E. A. Boyle
Massive releases of icebergs into the North Atlantic during the last glacial period, and the associated storminess, periodically mixed the ocean to greater depths than usual.

GENETICS

Wasp Gene Expression Supports an Evolutionary Link Between Maternal Behavior and Eusociality 441
A. L. Toth et al.
Analysis of a set of genes expressed in the brain of a primitive wasp shows that the care shown by worker wasps toward siblings probably evolved from maternal care behavior.

MOLECULAR BIOLOGY

JMJD6 Is a Histone Arginine Demethylase 444
B. Chang, Y. Chen, Y. Zhao, R. K. Bruick
An enzyme is discovered that removes methyl groups from arginines in histone proteins, which together with DNA form the bulk of chromatin and help regulate gene expression.
>> Perspective p. 403; Report p. 447

MOLECULAR BIOLOGY

Demethylation of H3K27 Regulates Polycomb Recruitment and H2A Ubiquitination 447
M. G. Lee et al.
An enzyme is described that removes methyl groups from a lysine in a human histone protein, a modification thought to help regulate gene expression. *>> Perspective p. 403; Report p. 444*

MOLECULAR BIOLOGY

Permuted tRNA Genes Expressed via a Circular RNA Intermediate in *Cyanidioschyzon merolae* 450
A. Soma et al.
RNA transcribed from split, noncontiguous transfer RNA genes in red algae is circularized, joining the discontinuous segments and allowing processing into mature transfer RNA.
>> Perspective p. 405; Brevia p. 415

PLANT SCIENCE

Trojan Horse Strategy in *Agrobacterium* Transformation: Abusing MAPK Defense Signaling 453
A. Djamei, A. Pitzschke, H. Nakagami, I. Rajh, H. Hirt
A bacterial plant pathogen co-opts one of the plant's own defense proteins to facilitate transfer of its infectious DNA into the nucleus.

MOLECULAR BIOLOGY

Structure of a NHEJ Polymerase–Mediated DNA Synaptic Complex 456
N. C. Brissett et al.
Broken, nonhomologous prokaryotic DNA is repaired by a DNA polymerase that enables each end to search for complementarity on the opposing end.

STRUCTURAL BIOLOGY

Structure of Hexameric DnaB Helicase and Its Complex with a Domain of DnaG Primase 459
S. Bailey, W. K. Eliason, T. A. Steitz
The structures of the two enzymes that initiate replication on single-stranded DNA show that one stimulates the other by stabilizing it as a hexamer in an activated conformation.

CELL BIOLOGY

Network Analysis of Oncogenic Ras Activation in Cancer 463
E. C. Stites, P. C. Trampont, Z. Ma, K. S. Ravichandran
A model of cell signaling reveals why only one mutated form of an oncogene occurs in tumors and suggests a strategy for selective inhibition of oncogene-containing cells.

ECOLOGY

Light-Responsive Cryptochromes from a Simple Multicellular Animal, the Coral *Acropora millepora* 467
O. Levy et al.
Primitive photoreceptors in a reef-building coral respond to subtle changes in light, suggesting that they help trigger the mass spawning of corals on the Great Barrier Reef.

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

SCIENCE (ISSN 0036-8075) is published weekly on Friday, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005. Periodicals Mail postage (publication No. 484460) paid at Washington, DC, and additional mailing offices. Domestic individual membership and subscription (51 issues): $142 ($74 allocated to subscription). Domestic institutional subscription (51 issues): $710; Foreign postage extra: Mexico, Caribbean (surface mail) $55; other countries (air assist delivery) $85. First class, airmail, student, and emeritus rates on request. Canadian rates with GST available upon request, GST #1254 88122. Publications Mail Agreement Number 1069624. SCIENCE is printed on 30 percent post-consumer recycled paper. **Printed in the U.S.A.**

Change of address: Allow 4 weeks, giving old and new addresses and 8-digit account number. **Postmaster:** Send change of address to AAAS, P.O. Box 96178, Washington, DC 20090–6178. **Single-copy sales:** $10.00 current issue, $15.00 back issue prepaid includes surface postage; bulk rates on request. The identification code for *Science* is 0036-8075. *Science* is indexed in the *Reader's Guide to Periodical Literature* and in several specialized indexes.

Printed on 30% post-consumer recycled paper.

CONTENTS continued >>

Gift of gab.

SCIENCENOW

www.sciencenow.org DAILY NEWS COVERAGE

Listening to Rumors
When evaluating someone's character, people believe gossip rather than other information.

Superduper Nova
Brightest blast from the past yet throws a kink in stellar explosion theory.

From Flu to Superflu
Single protein may explain why 1918 virus was so deadly.

Asparagine deamidation, a molecular timer?

SCIENCE'S STKE

www.stke.org
SIGNAL TRANSDUCTION KNOWLEDGE ENVIRONMENT

REVIEW: Chronoregulation by Asparagine Deamidation
S. J. Weintraub and B. E. Deverman
Asparagine deamidation may act as a genetically programmed molecular timer of biological processes.

ST NETWATCH: AVIS
AVIS is a Google gadget–compatible Web-based viewer of interactive cell signaling networks; in Modeling Tools.

ST NETWATCH: miRBase
miRBase contains databases of published miRNA sequences and predicted miRNA targets; in Bioinformatics Resources.

Opportunities at your business school.

SCIENCE CAREERS

www.sciencecareers.org CAREER RESOURCES FOR SCIENTISTS

US: Walking on the Dark Side
P. Fiske
Grad students and postdocs can find good opportunities at their local business schools.

US: Making a Midlife Change to Academia
A. Kotok
Why would a researcher walk away from two decades with a company to accept a new university post?

UK: Reality Check
L. Laursen
A new U.K. report on Ph.D. jobs finds no surprises but shows how many directions graduates can take.

GLOBAL: *Science* Careers Blog
Science *Careers Staff*
Get frequent updates from the science-career trenches, including advice, opinions, news, and funding.

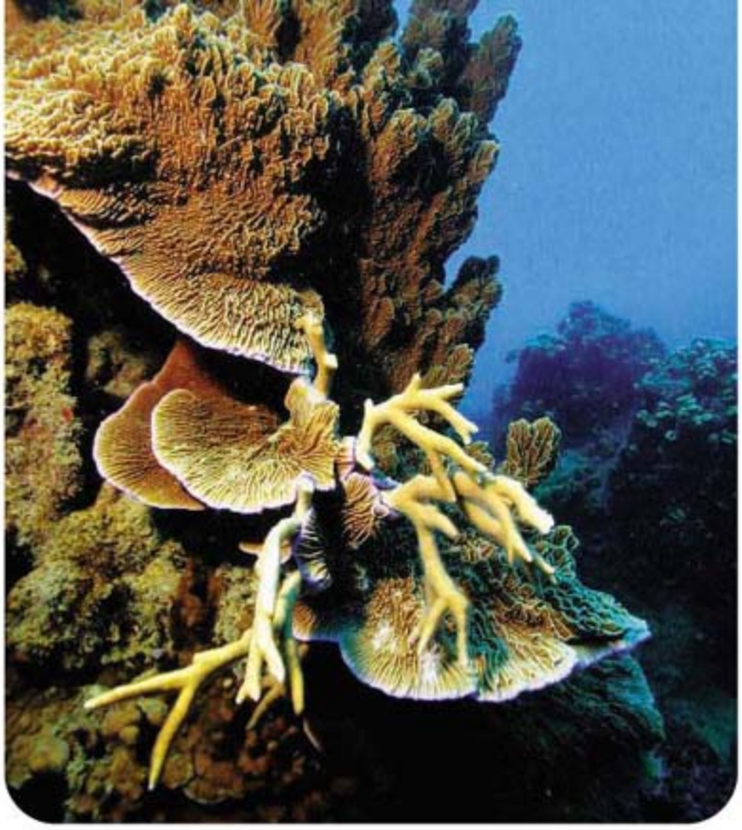
SCIENCEPODCAST

Download the 19 October *Science* Podcast to hear about how corals detect moonlight, the composition of solar wind, genetic ancestry testing, choosing sites for international facilities, and more.
www.sciencemag.org/about/podcast.dtl

CREDITS: (SCIENCENOW) STOCKBYTE; (SCIENCE CAREERS) TANBIR AHMED/CREATIVE COMMONS

Separate individual or institutional subscriptions to these products may be required for full-text access.

EDITED BY STELLA HURTLEY AND PHIL SZUROMI



<< Clocks in the Corals

Moonlight triggers the synchronized spawning of reef-building corals; however, the mechanism underlying detection of moonlight by these animals is largely unknown. **Levy *et al.*** (p. 467) now demonstrate the presence of ancient blue-light–sensing photoreceptors, cryptochromes, in the reef-building coral *Acropora millepora* (phylum Cnidaria). Cryptochromes regulate entrainment of the circadian clock of higher animals and plants. Expression of two coral cryptochrome genes, *cry1* and *cry2*, was rhythmic under a light-dark cycle but not in constant darkness. Expression of *cry2* varied with the full moon. This work suggests that cryptochromes not only function in the circadian clock of plants and higher animals, but may trigger the synchronized spawning of the Great Barrier Reef.

Insights into Wasp Eusociality

The observation that "worker" insects will care for their siblings rather than reproduce themselves is a hallmark of eusociality, a form of altruism that has long fascinated biologists, including Darwin. **Toth *et al.*** (p. 441, published online 27 September) tested the idea that this behavior evolved from early expression of "maternal care genes" prior to reproductive development in the wasp *Polistes metricus*. Unlike the better-studied honey bee, both workers and reproductives display maternal (brood provisioning) behavior in this wasp, but at different times. Similarities to the sequenced genome of the honey bee were used to identify a set of wasp genes expressed in the brain or known to be relevant to behavior. Reproductive, maternal females had gene expression patterns more like nonreproductive, maternal females (workers) than like reproductive, nonmaternal females (queens).

Finite, Huge, and Complex

Many physical systems, such as the atmosphere, transportation networks, and the Internet, are highly complex, and researchers have devoted much effort to modeling these systems mathematically. However, mathematics itself can also be a complex system, in which seemingly simple principles produce an exploding number of objects or structures. **Foote** (p. 410) reviews the case of finite group theory, which features such examples as the Enormous Theorem requiring 15,000 pages of proofs, and the Monster group containing 10^{54} elements. The ways in which mathematical concepts result in complex structures may help us understand the complexity of physical systems and vice versa.

Tracing Mercury's Movements

Mercury has many isotopes, and changes in its isotopic ratios may provide clues for tracing its movement in the environment. **Bergquist and Blum** (p. 417, published online 13 September; see the Perspective by **Lamborg**) now show that, in addition to the normal mass-dependent fractionation of isotopes that is typically seen, the odd isotopes of mercury under certain reduction conditions show evidence of a mass-independent fractionation (which has been shown previously for oxygen and sulfur). Through experiments in the lab and with fish from Lakes Michigan and Champlain, this isotopic signature was used to trace the loss of methylmercury by photoreduction.

From Mussels to Multiuse Coatings

Surface coatings often must be tailored to the substrates—hence we use different formulations to coat plaster walls versus wood trim. **H. Lee *et al.*** (p. 426) show that surfaces dipped into slightly basic dopamine solutions (inspired by the adhesives used by mussels) develop a polymeric coating that can be readily modified by secondary reactions. The polydopamine coating is generic and can be applied to surfaces of different materials (metals, polymers, and ceramics), as well as complex or patterned surfaces. The coated polydopamine surfaces can undergo two types of secondary reactions, such as metallization and self-assembled monolayer formation.

The Inside Scoop on Gold Nanoparticles

Metal nanoparticles are generally nonuniform and characterized by microscopy, but well-defined large metal clusters (more than 100 metal atoms) have been synthesized and characterized by x-ray diffraction. In the examples for platinum group metals, the metal-metal bonding is strong and dominates the packing of metal shells. Through a careful growth technique, **Jadzinsky *et al.*** (p. 430, see the cover and the Perspective by **Whetten**) obtained a crystalline sample of nanoparticles each containing 102 gold atoms and have solved the structure by x-ray diffraction to a resolution of 1.1 angstroms. The decahedral core geometry is consistent with prior hypotheses, but surface groups exert a strong influence on the outer gold shell and contribute to the electron count that stabilizes the cluster. The self-interactions of organosulfur-capping ligands create a rigid layer that imparts chirality to the clusters.

Continued on page 357

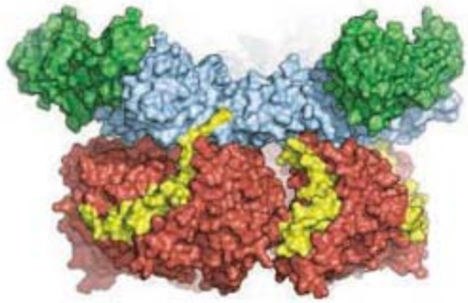
CREDITS (TOP TO BOTTOM): OVE HOEGH-GULDBERG; H. LEE *ET AL.*

Continued from page 355

Warming from the Cold Places

The details of how the different parts of the climate system act and interact during changes from glacial to interglacial states are still being resolved. **Stott *et al.*** (p. 435; published online 27 September; see the 28 September news story by **Kerr**) construct a chronology of high- and low-latitude climate change at the last glacial termination, in order to help answer the questions of where warming originated, and why. Their data, derived from both benthic and planktonic foraminifera recovered from the same marine sediment core, indicate that deep-sea temperatures in the western tropical Pacific warmed about 1500 years before the surface waters did, a result of the earlier warming of the high-latitude surface water from where the deep water originated. The deep-sea warming also preceded the rise in atmospheric CO_2, which suggests that increasing insolation at high southern latitudes caused a retreat of sea ice that led to warming there and further afield.

Unwinding and Priming DNA



Most DNA polymerases can only initiate DNA synthesis on a primed single-stranded (ss) DNA substrate. In eubacterial cells, DNA unwinding and priming is achieved by a complex of the DnaB helicase and the DnaG primase. The interaction between DnaB and DnaG stimulates both of their activities, but how this is achieved has been unclear. **Bailey *et al.*** (p. 459) report crystal structures of unliganded hexameric DnaB and its complex with the helicase binding domain (HBD) of DnaG. The two domains of DnaB pack with different symmetries to provide a two-layered ring structure. Three bound HBDs stabilize the hexamer in a conformation that may increase its processivity, and a potential ssDNA binding site on DnaB may guide the DNA to the DnaG active site.

More Different Than Expected

A method for identifying genomic structural variants (SVs, a type of variation including copy-number variants) is described by **Korbel *et al.*** (p. 420, published online 27 September). Paired-end mapping can quickly identify the location of the breakpoints at high resolution and determine in most cases exactly where in the genome they occur. With this method, the analysis of DNA from two individuals of different ethnic backgrounds shows unexpected amounts of SVs between individuals, which indicates that people are more genetically diverse than previously realized. Among the important findings is the observation that SVs are associated with certain (but not all) types of repeats, as well as unique sequences; insights also emerge into mechanisms by which SVs arise.

Off with the Methyl Marks

The methylation of histones, proteins that make up the bulk of chromatin in eukaryotes, plays a critical role in the epigenetic regulation of gene expression. Although the enzymes that put this mark onto chromatin are well known, the class of enzymes that take it off again, the Jumonji C (JmjC) family of demethylases, are a more recent discovery (see the Perspective by **Rivenbark and Strahl**). Although several JmjC lysine demethylases are known, no JmjC protein has been identified that can remove methyl groups from arginine residues in histones. **Chang *et al.*** (p. 444) now report the discovery of an enzyme, JMJD6, that demethylates histone H3 at arginine 2 and histone H4 at arginine 3, marks that are likely a critical part of the "histone code" that modulates chromatin function. Di- and trimethylation of histone H3 on lysine 27 (H3K27me2-3) are exclusively repressing signals and are implicated in X-chromosome inactivation, imprinting, stem cell maintenance, circadian rhythms, and cancer. The enzyme that places the marks has been known, and now **M. G. Lee *et al.*** (p. 447, published online 30 August) have identified the human enzyme, UTX (ubiquitously transcribed mouse X-chromosome gene), a JmjC domain–containing protein (similar to other demethylase enzymes), responsible for removing the H3K27me2-3 marks and promoting the activation of gene expression.

CREDIT: BAILEY ET AL.

Norman Borlaug was awarded the Nobel Peace Prize in 1970. Since 1986, he has been engaged with Jimmy Carter and the Nippon Foundation of Japan in African agricultural development and is currently president of the Sasakawa Africa Association.

Feeding a Hungry World

NEXT WEEK, MORE THAN 200 SCIENCE JOURNALS THROUGHOUT THE WORLD WILL simultaneously publish papers on global poverty and human development—a collaborative effort to increase awareness, interest, and research about these important issues of our time. Some 800 million people still experience chronic and transitory hunger each year. Over the next 50 years, we face the daunting job of feeding 3.5 billion additional people, most of whom will begin life in poverty. The battle to alleviate poverty and improve human health and productivity will require dynamic agricultural development.

Breakthroughs in wheat and rice production, which came to be known as the Green Revolution, signaled the dawn of applying agricultural science to the Third World's need for modern techniques. It began in Mexico in the late 1950s, spread to Asia during the 1960s and 1970s, and continued in China in the 1980s and 1990s. Over a 40-year period, the proportion of hungry people in the world declined from about 60% in 1960 to 17% in 2000. The Green Revolution also brought environmental benefits. If the global cereal yields of 1950 still prevailed in 2000, we would have needed nearly 1.2 billion more hectares of the same quality, instead of the 660 million hectares used, to achieve 2000's global harvest. Moreover, had environmentally fragile land been brought into agricultural production, the soil erosion, loss of forests and grasslands, reduction in biodiversity, and extinction of wildlife species would have been disastrous.

Today, nearly two-thirds of the world's hungry people are farmers and pastoralists who live in marginal lands in Asia and Africa, where agro-climatic stresses and/or extreme remoteness make agricultural production especially risky and costly. Africa has been the region of greatest concern. High rates of population growth and little application of improved production technology during the past three decades have resulted in declining per capita food production, escalating food deficits, deteriorating nutritional levels among the rural poor, and devastating environmental degradation. There are signs that smallholder food production may be turning around through the application of science and technology to basic food production, but this recovery is still fragile. But African capacity in science and technology needs strengthening, and massive investments in infrastructure are required, especially for roads and transport, potable water, and electricity.

For the foreseeable future, plants—especially the cereals—will continue to supply much of our increased food demand, both for direct human consumption and as livestock feed to satisfy the rapidly growing demand for meat in the newly industrializing countries. The demand for cereals will probably grow by 50% over the next 20 years, and even larger harvests will be needed if more grain is diverted to produce biofuels. Seventy percent of global water withdrawals are for irrigating agricultural lands, which contribute 40% of our global food harvest. Expanding irrigated areas will be critical to meet future food demand, but expansion must be accompanied by greater efficiencies in water management.

Although sizable land areas, such as the cerrados of Brazil, may responsibly be converted to agriculture, most food increases will have to come from lands already in production. Fortunately, productivity improvements in crop management can be made all along the line: in plant breeding, crop management, tillage, fertilization, weed and pest control, harvesting, and water use. Genetically engineered crops are playing an increasingly important role in world agriculture, enabling scientists to reach across genera for useful genes to enhance tolerance to drought, heat, cold, and waterlogging, all likely consequences of global warming. I believe biotechnology will be essential to meeting future food, feed, fiber, and biofuel demand.

The battle to ensure food security for hundreds of millions of miserably poor people is far from won. We must increase world food supplies but also recognize the links between population growth, food production, and environmental sustainability. Without a better balance, efforts to halt global poverty will grind to a halt.

– Norman Borlaug

10.1126/science.1151062

CREDITS: (ABOVE) MICHAEL COLLOPY; (RIGHT) WOLFGANG RATTAY/REUTERS

EDITED BY STELLA HURTLEY AND JAKE YESTON

ECOLOGY/EVOLUTION

INS AND OUTS OF EXTINCTION

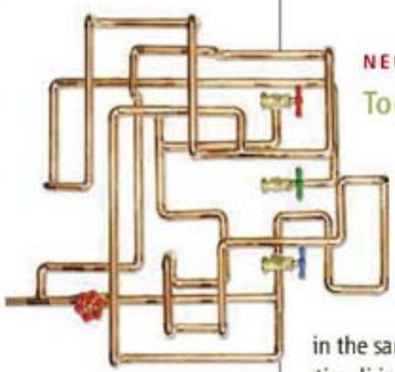
The global extinction of a species is the end point of a series of smaller-scale local extinctions of populations. Hence, the causes of extinction can be understood by studying patterns of extinction at the local scale. Species vary in their intrinsic vulnerability to extinction, and there is a range of extrinsic factors that can influence a population's survival; the probability of extinction might depend on the interplay of these two broad considerations. To study these questions, Fréville *et al.* took advantage of the Park Grass experiment, in which the fate of populations of herbaceous plants subjected to different fertilizer treatments have been followed for 60 years at a site in southeast England. The interactions of 11 intrinsic factors (life-history traits relating, for example, to reproduction and growth) with four extrinsic factors (such as nitrogen enrichment and acidification) were investigated. It transpired that population extinction could in most cases be related to the interaction of just one life-history trait with one extrinsic factor, but that the pairs of factors differed in different species. These findings point the way to a more accurate and predictive science of extinction, which will in turn provide a new tool for conservation managers attempting to reduce the rate of local extinctions caused by human activity. — AMS

Ecology **88**, 2662 (2007).

CHEMISTRY

Pulling Copper Along

Copper is a common choice for constructing pipes that carry drinking water because of its relatively strong resistance to corrosion, but over time oxidative chemistry can introduce metal ions into the streams emerging from the faucet. A complex series of factors contributes to the ion concentration, ranging from the water's pH to the precipitation equilibria of various hydroxide, oxide, and carbonate salts, as well as biochemical processes that accompany the formation of bacterial biofilms on the pipes' inner surfaces. In general though, a simplifying assumption has been that the aqueous copper ion concentration is limited by diffusion during stagnant periods between flow, when water rests in the pipe. Calle *et al.* have now found that the influence of flow dynamics cannot in general be neglected. Through a series of measurements on a pipe system connecting a well to a household in Chile, they uncovered concentration patterns suggesting that a significant quantity of ions is dislodged from surface biofilms by virtue of interfacial forces arising during flow. Thus, the interplay of hydrodynamics with sorption equilibria in these systems merits further study. — JSY



Environ. Sci. Technol. **41** 10.1021/es071079b (2007).

NEUROSCIENCE

Too Quick to Glimpse?

An optical illusion can help define which parts of the brain are responsible for human consciousness. People cannot consciously perceive a number flashed on a screen for 16 ms if it is quickly followed by another stimulus in the same area. As the time between the two stimuli increases, the first stimulus becomes visible; that is, it is accessible to the person's consciousness. Del Cul *et al.* recorded electrical brain waves from people's scalps as they were shown these stimuli and reported to the investigators whether they were visible or invisible. One brain wave in particular, P3, occurring 270 to 400 ms after the beginning of the trial, correlated with conscious perception of the stimulus. This wave seems to arise from sudden simultaneous activity in several parts of the brain, specifically the frontal, parietal, and temporal cortices of both hemispheres. These data are inconsistent with several proposed correlates of consciousness, including the rapid induced activity in the visual areas of the brain and the later more distributed, but still local, neural reverberations. Rather, they suggest that conscious perception is associated with a sudden global reverberation of neural activity, about 300 ms after the stimulus, encompassing several cortical areas bilaterally. — KK

PLoS Biol. **5**, 10.1371/journal.pbio.0050260 (2007).

GEOLOGY

Heat Bursts in the Highlands

Because rocks are good insulators, it is generally thought that temperatures deep in the crust evolve slowly, rising and falling over millions to tens of millions of years. Rapid pulses of fluid or the intrusion of hot magmas can heat or cool rocks more quickly, as can rapid uplift along a fault (which juxtaposes hot and cold rocks at a rate faster than heat conduction). Thus metamorphic processes are also thought to act over these time scales. Ague and Baxter challenge some of these notions in well-studied metamorphic rocks in Scotland, known as the Barrovian metamorphic belt and thought to represent bur-

Continued on page 363

CREDITS (TOP TO BOTTOM): M. DODD/AMANTA PHOTOLIBRARY; PAUL TAYLOR/GETTY IMAGES

Continued from page 361

ial and heating of rocks during continental collision. They show that concentrations of a trace element, strontium, across the mineral apatite are surprisingly variable. Laboratory data imply that if the minerals were at the temperatures inferred for the host rocks for even 1 million years, diffusion should have homogenized any gradients. Thus the authors infer that the rocks were heated and cooled in less time. This would seem to require rapid heat input by fluids and rapid exhumation, but at scales and rates that start to challenge what have been thought to be geologic limits. Stay tuned. — BH

Earth Planet. Sci. Lett. **261**, 500 (2007).

CHEMISTRY

A Light Switch in SWNTs

Although most realizations of molecular electronics make use of metallic leads, single-walled carbon nanotubes (SWNTs) can also serve as contacts. Oxidative cutting leaves carboxylate-decorated ends that can be covalently linked to diamine molecules so that the SWNT is reconnected through the molecule via amide linkages. Whalley *et al.* now use this approach to study ethene-bridged dithiophene and dipyrrole derivatives that photoconvert from ring-opened to ring-closed forms. Ultraviolet irradiation of the ring-opened thiophene derivative created a conjugated ring-closed form that was 25 times more conductive. Unlike the case for molecules bridging gold break-junction electrodes, neither visible light exposure nor heating recovered the open isomer, which the authors attribute to the greater energy dissipation from the excited state in this system. The pyrrole derivative could be thermally switched back, and showed a staircase rise in conductance with ultraviolet light exposure that was attributed to the presence of several molecules bridging the SWNT gap. — PDS

J. Am. Chem. Soc. **129**, 10.1021/ja073127y (2007).

ECOLOGY

Kelp in the Depths

Kelp forests are exceptionally productive marine ecosystems, iconic of high-latitude, shallow, cold waters. There are a few rare records of tropical deep-water species, but these are thought to be relicts of glacial-era populations. Graham *et al.* suggest that kelp may not be as restricted in distribution as once thought. By modeling the coincidence of the water temperature, bottom depth, and light penetration with nutrient circulation, they derive a map of potential tropical kelp beds, a rough contour of 25 to 236 m. A quick look offshore of the Galapagos Islands indeed revealed kelp at around 60 m depth. The authors also predict extensive kelp forests off Brazil, West Africa, and the Malay Archipelago. Essentially, wherever clear tropical water allows light to penetrate into cooler depths and bathymetries allow nutrient upwelling, kelp should survive in the tropics. Hence, even in strong El Niño years, tropical kelp can escape surface warming. — CA

Proc. Natl. Acad. Sci. U.S.A. **104**, 10.1073/pnas.0704778104 (2007).

Science STKE AAAS

www.stke.org

<< How Clocks See the Light

The circadian clocks that regulate daily rhythms in various processes in living organisms are entrained to a 24-hour cycle by mechanisms that detect daily changes in the amount of light in the environment. Hirayama *et al.* show that hydrogen peroxide (H_2O_2) can function as a required signaling molecule to transmit the sensation of light to changes in timing of the biochemical clock. In zebrafish, oscillators present in peripheral tissues and organs are sensitive to exposure to light. The authors used Z3 cells to show that exposure of the cells to light caused increased production of H_2O_2. Exposure of the cells to H_2O_2 increased expression of zebrafish *Cryptochrome* and *Period* genes (which encode components of the core clock machinery) with a time course similar to that observed when cells were exposed to light. Catalase is an antioxidant enzyme that can degrade H_2O_2, and the authors confirmed that light exposure stimulated expression of the *zCat* gene, but did so with a delayed time course consistent with its possible function in a negative feedback loop to cyclically suppress expression of the clock genes that initially resulted from light-induced generation of H_2O_2. In mammalian cells, H_2O_2 did not influence the expression of the clock genes, but mammalian peripheral tissues are not responsive to light. The identity of the phototransducer in the zebrafish system remains unknown. — LBR

Proc. Natl. Acad. Sci. U.S.A. **104**, 15747 (2007).

CREDIT: DR. SEAN CONNELL, UNIVERSITY OF ADELAIDE

www.sciencemag.org

Science

1200 New York Avenue, NW
Washington, DC 20005
Editorial: 202-326-6550, FAX 202-289-7562
News: 202-326-6581, FAX 202-371-9227

Bateman House, 82-88 Hills Road
Cambridge, UK CB2 1LQ
+44 (0) 1223 326500, FAX +44 (0) 1223 326501

SUBSCRIPTION SERVICES For change of address, missing issues, new orders and renewals, and payment questions: 866-434-AAAS (2227) or 202-326-6417, FAX 202-842-1065. Mailing addresses: AAAS, P.O. Box 96178, Washington, DC 20090-6178 or AAAS Member Services, 1200 New York Avenue, NW, Washington, DC 20005

INSTITUTIONAL SITE LICENSES please call 202-326-6755 for any questions or information

REPRINTS: Author Inquiries 800-635-7181
Commercial Inquiries 803-359-4578

PERMISSIONS 202-326-7074, FAX 202-682-0816z

MEMBER BENEFITS AAAS/Barnes&Noble.com bookstore www.aaas.org/bn; AAAS Online Store http://www.apisource.com/aaas/ code MKB6; AAAS Travels: Betchart Expeditions 800-252-4910; Apple Store www.apple/eppstore/aaas; Bank of America MasterCard 1-800-833-6262 priority code FAA3YU; Cold Spring Harbor Laboratory Press Publications www.cshlpress.com/affiliates/aaas.htm; GEICO Auto Insurance www.geico.com/landingpage/go51.htm?logo=17624; Hertz 800-654-2200 CDP#343457; Office Depot https://bsd.officedepot.com/portalLogin.do; Seabury & Smith Life Insurance 800-424-9883; Subaru VIP Program 202-326-6417; VIP Moving Services http://www.vipmayflower.com/domestic/index.html; Other Benefits: AAAS Member Services 202-326-6417 or www.aaasmember.org.

science_editors@aaas.org (for general editorial queries)
science_letters@aaas.org (for queries about letters)
science_reviews@aaas.org (for returning manuscript reviews)
science_bookrevs@aaas.org (for book review queries)

Published by the American Association for the Advancement of Science (AAAS), *Science* serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science, including the presentation of minority or conflicting points of view, rather than by publishing only material on which a consensus has been reached. Accordingly, all articles published in *Science*—including editorials, news and comment, and book reviews—are signed and reflect the individual views of the authors and not official points of view adopted by the AAAS or the institutions with which the authors are affiliated.

AAAS was founded in 1848 and incorporated in 1874. Its mission is to advance science and innovation throughout the world for the benefit of all people. The goals of the association are to: foster communication among scientists, engineers and the public; enhance international cooperation in science and its applications; promote the responsible conduct and use of science and technology; foster education in science and technology for everyone; enhance the science and technology workforce and infrastructure; increase public understanding and appreciation of science and technology; and strengthen support for the science and technology enterprise.

INFORMATION FOR AUTHORS

See pages 120 and 121 of the 5 January 2007 issue or access www.sciencemag.org/feature/contribinfo/home.shtml

EDITOR-IN-CHIEF **Donald Kennedy**
EXECUTIVE EDITOR **Monica M. Bradford**
DEPUTY EDITORS **R. Brooks Hanson, Barbara R. Jasny, Katrina L. Kelner**
NEWS EDITOR **Colin Norman**

EDITORIAL SUPERVISORY SENIOR EDITOR Phillip D. Szuromi; **SENIOR EDITOR/PERSPECTIVES** Lisa D. Chong; **SENIOR EDITORS** Gilbert J. Chin, Pamela J. Hines, Paula A. Kiberstis (Boston), Marc S. Lavine (Toronto), Beverly A. Purnell, L. Bryan Ray, Guy Riddihough, H. Jesse Smith, Valda Vinson, David Voss; **ASSOCIATE EDITORS** Jake S. Yeston, Laura M. Zahn; **ONLINE EDITOR** Stewart Wills; **ASSOCIATE ONLINE EDITORS** Robert Frederick, Tara S. Marathe; **BOOK REVIEW EDITOR** Sherman J. Suter; **ASSOCIATE LETTERS EDITOR** Jennifer Sills; **EDITORIAL MANAGER** Cara Tate; **SENIOR COPY EDITORS** Jeffrey E. Cook, Cynthia Howe, Harry Jach, Barbara P. Ordway, Trista Wagoner; **COPY EDITORS** Lauren Kmec, Peter Mooreside; **EDITORIAL COORDINATORS** Carolyn Kyle, Beverly Shields; **PUBLICATIONS ASSISTANTS** Ramatoulaye Diop, Chris Filiatreau, Joi S. Granger, Jeffrey Hearn, Lisa Johnson, Scott Miller, Jerry Richardson, Brian White, Anita Wynn; **EDITORIAL ASSISTANTS** Emily Guise, Patricia M. Moore, Jennifer A. Seibert; **EXECUTIVE ASSISTANT** Sylvia S. Kihara; **ADMINISTRATIVE SUPPORT** Maryrose Madrid

NEWS SENIOR CORRESPONDENT Jean Marx; **DEPUTY NEWS EDITORS** Robert Coontz, Eliot Marshall, Jeffrey Mervis, Leslie Roberts; **CONTRIBUTING EDITORS** Elizabeth Culotta, Polly Shulman; **NEWS WRITERS** Yudhijit Bhattacharjee, Adrian Cho, Jennifer Couzin, David Grimm, Constance Holden, Jocelyn Kaiser, Richard A. Kerr, Eli Kintisch, Andrew Lawler (New England), Greg Miller, Elizabeth Pennisi, Robert F. Service (Pacific NW), Erik Stokstad; **INTERN** Benjamin Lester; **CONTRIBUTING CORRESPONDENTS** Barry A. Cipra, Jon Cohen (San Diego, CA), Daniel Ferber, Ann Gibbons, Robert Irion, Mitch Leslie, Charles C. Mann, Evelyn Strauss, Gary Taubes; **COPY EDITORS** Rachel Curran, Linda B. Felaco, Melvin Gatling; **ADMINISTRATIVE SUPPORT** Scherraine Mack, Fannie Groom; **BUREAUS** New England: 207-549-7755, San Diego, CA: 760-942-3252, FAX 760-942-4979, Pacific Northwest: 503-963-1940

PRODUCTION DIRECTOR James Landry; **SENIOR MANAGER** Wendy K. Shank; **ASSISTANT MANAGER** Rebecca Doshi; **SENIOR SPECIALISTS** Jay Covert, Chris Redwood; **SPECIALIST** Steve Forrester; **PREFLIGHT DIRECTOR** David M. Tompkins; **MANAGER** Marcus Spiegler; **SPECIALIST** Jessie Mudjitaba

ART DIRECTOR Kelly Buckheit Krause; **ASSOCIATE ART DIRECTOR** Aaron Morales; **ILLUSTRATORS** Chris Bickel, Katharine Sutliff; **SENIOR ART ASSOCIATES** Holly Bishop, Laura Creveling, Preston Huey, Nayomi Kevitiyagala; **ASSOCIATE** Jessica Newfield; **PHOTO EDITOR** Leslie Blizard

***SCIENCE* INTERNATIONAL**

EUROPE (science@science-int.co.uk) **EDITORIAL: INTERNATIONAL MANAGING EDITOR** Andrew M. Sugden; **SENIOR EDITOR/PERSPECTIVES** Julia Fahrenkamp-Uppenbrink; **SENIOR EDITORS** Caroline Ash, Stella M. Hurtley, Ian S. Osborne, Stephen J. Simpson, Peter Stern; **ASSOCIATE EDITOR** Joanne Baker; **EDITORIAL SUPPORT** Deborah Dennison, Rachel Roberts, Alice Whaley; **ADMINISTRATIVE SUPPORT** Janet Clements, Jill White; **NEWS: EUROPE NEWS EDITOR** John Travis; **DEPUTY NEWS EDITOR** Daniel Clery; **CONTRIBUTING CORRESPONDENTS** Michael Balter (Paris), John Bohannon (Vienna), Martin Enserink (Amsterdam and Paris), Gretchen Vogel (Berlin); **INTERN** Elizabeth Quill

ASIA Japan Office: Asca Corporation, Eiko Ishioka, Fusako Tamura, 1-8-13, Hirano-cho, Chuo-ku, Osaka-shi, Osaka, 541-0046 Japan; +81 (0) 6 6202 6272, FAX +81 (0) 6 6202 6271; asca@os.gulf.or.jp; **ASIA NEWS EDITOR** Richard Stone (Beijing: rstone@aaas.org); **CONTRIBUTING CORRESPONDENTS** Dennis Normile (Japan: +81 (0) 3 3391 0630, FAX 81 (0) 3 5936 3531; dnormile@gol.com); Hao Xin (China: + 86 (0) 10 6307 4439 or 6307 3676, FAX +86 (0) 10 6307 4358; cindyhao@gmail.com); Pallava Bagla (South Asia: +91 (0) 11 2271 2896; pbagla@vsnl.com)

AFRICA Robert Koenig (contributing correspondent, rob.koenig@gmail.com)

EXECUTIVE PUBLISHER **Alan I. Leshner**
PUBLISHER **Beth Rosner**

FULFILLMENT SYSTEMS AND OPERATIONS (membership@aaas.org) **DIRECTOR** Waylon Butler; **CUSTOMER SERVICE SUPERVISOR** Pat Butler; **SPECIALISTS** Laurie Baker, Latoya Casteel, Lavanda Crawford, Vicki Linton; **DATA ENTRY SUPERVISOR** Cynthia Johnson; **SPECIALISTS** Tomeka Diggs, Tarrika Hill, Erin Layne, Sheila Thomas; **SYSTEMS ANALYST** Tim Popoola

BUSINESS OPERATIONS AND ADMINISTRATION DIRECTOR Deborah Rivera-Wienhold; **ASSISTANT DIRECTOR, BUSINESS OPERATIONS** Randy Yi; **SENIOR FINANCIAL ANALYSTS** Michael LoBue, Jessica Tierney; **FINANCIAL ANALYSTS** Nicole Nicholson, Farida Yeasmin; **RIGHTS AND PERMISSIONS: ADMINISTRATOR** Emilie David; **ASSOCIATE** Elizabeth Sandler; **MARKETING DIRECTOR** John Meyers; **MARKETING MANAGERS** Darryl Walter, Allison Pritchard; **MARKETING ASSOCIATES** Julianne Wielga, Mary Ellen Crowley, Alison Chandler, Marcia Leach, Wendy Wise; **INTERNATIONAL MARKETING MANAGER** Wendy Sturley; **MARKETING EXECUTIVE** Jennifer Reeves; **MARKETING/MEMBER SERVICES EXECUTIVE** Linda Rusk; **JAPAN SALES** Jason Hannaford; **SITE LICENSE SALES DIRECTOR** Tom Ryan; **SALES MANAGER** Russ Edra; **SALES AND CUSTOMER SERVICE** Mehan Dossani, Iquo Edim, Kiki Forsythe, Catherine Holland, Phillip Smith, Philip Tsolakidis; **ELECTRONIC MEDIA: MANAGER** Lizabeth Harman; **PROJECT MANAGER** Trista Snyder; **ASSISTANT MANAGER** Lisa Stanford; **SENIOR PRODUCTION SPECIALIST** Walter Jones; **PRODUCTION SPECIALISTS** Nichele Johnston, Kimberly Oster

ADVERTISING DIRECTOR WORLDWIDE AD SALES Bill Moran

PRODUCT (science_advertising@aaas.org); **CONSUMER & SPONSORSHIP SALES MANAGER** Tina Morra: 202-326-6542; **MIDWEST** Rick Bongiovanni: 330-405-7080, FAX 330-405-7081; **WEST COAST/W. CANADA** Teola Young: 650-964-2266; **EAST COAST/E. CANADA** Christopher Breslin: 443-512-0330, FAX 443-512-0331; **UK/EUROPE/ASIA** Michelle Field: +44 (0) 1223-326-524, FAX +44 (0) 1223-325-532; **JAPAN** Mashy Yoshikawa: +81 (0) 33235 5961, FAX +81 (0) 33235 5852; **SENIOR TRAFFIC ASSOCIATE** Deiandra Simms

COMMERCIAL EDITOR Sean Sanders: 202-326-6430

CLASSIFIED (advertise@sciencecareers.org); **US: RECRUITMENT SALES MANAGER** Ian King: 202-326-6528, FAX 202-289-6742; **INSIDE SALES MANAGER: MIDWEST/CANADA** Daryl Anderson: 202-326-6543; **US/INDUSTRY** Allison Millar: 202-326-6572; **NORTHEAST** Alexis Fleming: 202-326-6578; **SOUTHEAST** Tina Burks: 202-326-6577; **WEST** Nicholas Hintibidze: 202-326-6533; **SALES COORDINATORS** Erika Foard, Rohan Edmonson, Shirley Young; **INTERNATIONAL: SALES MANAGER** Tracy Holmes: +44 (0) 1223 326525, FAX +44 (0) 1223 326532; **SALES** Marium Hudda, Alex Palmer, Alessandra Sorgente; **SALES ASSISTANT** Louise Moore; **JAPAN** Jason Hannaford: +81 (0) 52 757 5360, FAX +81 (0) 52 757 5361; **ADVERTISING PRODUCTION OPERATIONS MANAGER** Deborah Tompkins; **SENIOR PRODUCTION SPECIALISTS** Robert Buck, Amy Hardcastle; **SENIOR TRAFFIC ASSOCIATE** Christine Hall; **PUBLICATIONS ASSISTANT** Mary Lagnaoui

AAAS BOARD OF DIRECTORS **RETIRING PRESIDENT, CHAIR** John P. Holdren; **PRESIDENT** David Baltimore; **PRESIDENT-ELECT** James J. McCarthy; **TREASURER** David E. Shaw; **CHIEF EXECUTIVE OFFICER** Alan I. Leshner; **BOARD** John E. Dowling, Lynn W. Enquist, Susan M. Fitzpatrick, Alice Gast, Linda P. B. Katehi, Cherry A. Murray, Thomas D. Pollard, Kathryn D. Sullivan

AAAS
ADVANCING SCIENCE, SERVING SOCIETY

Peterson, coldly pursuing a new mineral.

Rock Candy For Martians?

A chilly wilderness quest has led a Canadian researcher to a new mineral that may be more at home on Mars. Ronald Peterson, a mineralogist at Queen's University in Kingston, Canada, first suspected the existence of the exotic substance in 2005, when the Mars rover Opportunity kicked up magnesium sulfate dust and photographed lens-shaped holes in sedimentary rocks on the cratered plain of Meridiani Planum. Peterson and his colleague Ruiyao Wang posited that the rover had spotted evidence of a novel platelike form of magnesium sulfate—a low-temperature cousin of Epsom salts, with 11 water molecules in its structure instead of the usual seven. To make the case for the mineral, Peterson and colleagues set out to find it on Earth.

Near the shore of a frozen-over lake in British Columbia, Peterson spotted kilograms of snowy off-white crystals growing amid the shredded bark of a dead tree trunk. The wood fibers had wicked up water along with magnesium and sulfate from old mines nearby. The team packed up samples on dry ice, and Peterson rushed them back to his lab. Working outside to keep the samples cold, he examined the crystals under a microscope and later confirmed their structure by x-ray diffraction. The mineral, christened meridianiite, is described in the October issue of *American Mineralogist*.

"New minerals that we find are usually tiny fly specks," Peterson says. "It's unusual to find one in kilograms." Mars's polar ice caps might harbor much more of the mineral, he says.

CREDITS (TOP TO BOTTOM): QUEEN'S UNIVERSITY; TONY LINCK/TIME & LIFE PICTURES/GETTY IMAGES; SPACEART

Tongues Untied

Drugs may boost old-school therapy for stutterers.

A new center at the University of California, Irvine, is the first to specialize in using drugs to treat stuttering.

Stuttering affects approximately 1% of the adult population worldwide, says Gerald Maguire, the psychiatrist heading the new center, which opened 4 October. (Even the ancient Egyptians had stutterers among their ranks—and a hieroglyph to depict the condition.) Speech therapy is standard treatment, but it tends to help children more than adults, Maguire says.

Although no drug has been approved by the U.S. Food and Drug Administration for treating stuttering, a handful of studies have suggested that off-label use of dopamine-blocking antipsychotic drugs can be helpful, Maguire says. He has taken low doses of the antipsychotic drug olanzapine for almost 10 years to treat his own stutter. "Now my speech is more automatic," he says. "I used to be constantly anxious, constantly monitoring my words."

Patients at the clinic could also elect to enroll in a clinical trial to test the stutter-stopping ability of pagoclone, a drug that boosts activity of the neurotransmitter GABA. (Maguire acknowledges receiving consulting fees and research support from the company that makes the drug as well as from Eli Lilly and Co., the maker of olanzapine.) "It's a great thing to try," says Dennis Drayna, a geneticist who studies stuttering at the National Institute on Deafness and Other Communication Disorders in Bethesda, Maryland. Researchers have long sought drugs to treat stuttering, with mixed results, Drayna says, but given its prevalence, "a good pharmacological therapy would be a great advance."

Into the Woods

NET WATCH

Looking for historical maps of Spanish woodlands? Curious about which invasive species have put down roots in Estonia's boreal forest? Drop by the newly sprouted Euroforest Portal, from the European Forest Institute and Finland's University of Joensuu.

Visitors will find hundreds of annotated links to forest information for more than 40 countries. You can check the results of Germany's most recent forest inventory, browse an atlas of Russia's remaining pristine forests, or read a World Wildlife Fund report on Europe's involvement in the illegal logging trade. For students, the site also lists opportunities for research and training in forestry. >>
forestportal.efi.int

Fending Off a Killer

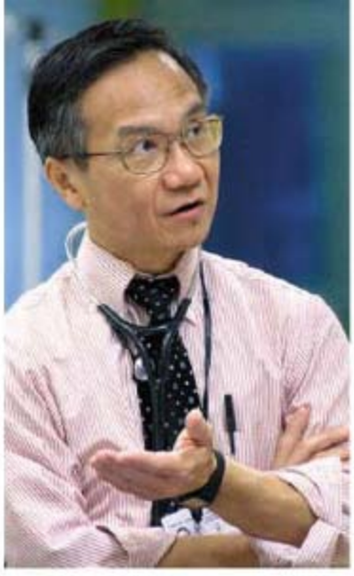
Boys have amused themselves for ages burning holes in leaves—or roasting the odd ant—by concentrating sunlight through a hand lens. The same technique may someday save civilization from destruction. A research team at the University of Glasgow in the U.K. has analyzed nine methods proposed for deflecting an asteroid from a collision course with Earth. The winner: concentrating sunlight on the asteroid to create a jet of hot gas that would nudge it off course.

"Mirror bees" sting an Earth-menacing intruder.

The Glasgow group, led by space systems engineer Massimiliano Vasile, considered everything from hitting the asteroid with a speeding projectile to mounting a rocket on it. Most practical were asteroid-orbiting light-focusing mirrors and nearby nuclear blasts, they concluded in a presentation early this month. "We preferred the solar solution," says Vasile. "It's as effective as nuclear and less risky," one risk with nuclear being shattering the target into debris that could then strike Earth like a shotgun blast.

A swarm of 20-meter "mirror bees" could be launched within 20 years, the group says. The most important lesson from the work, says planetary physicist Jay Melosh of the University of Arizona, Tucson, is "to realize there are viable non-nuclear options for deflecting asteroids."

EDITED BY YUDHIJIT BHATTACHARJEE



Pioneers

NOT ALONE. Dozens of parents whose children suffer from neuroblastoma, a rare and deadly childhood cancer, have banded together to fund a drug development effort at Memorial Sloan-Kettering Cancer Center (MSKCC) in New York City. The idea came out of a meeting this summer between patients' families and MSKCC pediatric oncologist Nai-Kong Cheung (left), who more than 20 years ago developed a therapy for the disease, a mouse antibody called 3F8. Answering questions about the most urgent needs in neuroblastoma, Cheung pointed out that humanizing the antibody—replacing the mouse genes in the antibody blueprint with human ones—would reduce immune resistance to the therapy. "I told them, get organized and raise money to help," Cheung says.

Last month, the fundraising effort got under way as seven fathers of children with neuroblastoma completed a cross-country bike ride dubbed "The Loneliest Road." It netted $200,000. More than 60 families have formed a group called Band of Parents to raise the $2 to $3 million needed for the project. "From a grants standpoint … there's no discovery aspect" to humanizing 3F8, making it unappealing to government funders, says Thomas Melgar, a physician whose 6-year-old son Austin has neuroblastoma and who is on the Band of Parents executive committee. "We want to be involved," he says, in determining what type of neuroblastoma research is pursued.

CHECKING IN

NEW HOME, NEW PURPOSE. The new president of the Human Genome Organisation (HUGO) says the 18-year-old international group should try to find common ground on pressing privacy and ethical issues now that the human genome has been sequenced. Edison Liu, a noted cancer researcher who directs the Genome Institute of Singapore, began his 3-year-term this summer and recently initiated HUGO's move from London to Singapore.

"HUGO has to have a new role," says Liu, who served as director of clinical sciences at the National Cancer Institute in Bethesda, Maryland, before founding the Singapore institute in 2001. He says increasing scientific capabilities means that the developing nations of Asia and Latin America will not only benefit from but also contribute to the rapid advances in genomic medicine.

MONEY MATTERS

SHARING GOOD LUCK. A billionaire cancer survivor is putting $100 million into a new institute at the Massachusetts Institute of Technology (MIT) in Cambridge to help geneticists, molecular biologists, and engineers combat the disease.

David Koch, an MIT grad, is executive vice president of Koch Industries Inc., an industrial powerhouse in the chemical, mining, timber, and banking fields. Koch, 67, has an estimated net worth of $17 billion, good for ninth place on *Forbes*' list of the 400 richest Americans. He also has a political streak, running unsuccessfully as the vice presidential candidate for the Libertarian Party in 1980.

The David H. Koch Institute for Integrative Cancer Research will open in 2010 and will be led by MIT biologist Tyler Jacks. More than two dozen researchers and engineers will work together on new therapies and advanced diagnostics. "As a cancer survivor, I feel especially fortunate to be able to help advance" efforts to conquer the disease, says Koch, who was diagnosed with prostate cancer 15 years ago.

Got a tip for this page? E-mail people@aaas.org

CREDITS (TOP TO BOTTOM): JULIANA THOMAS FOR THE PHOTOGRAPH; LARRY KIRK; D. NORMILE/SCIENCE; UNIVERSITY OF HELSINKI, FINLAND

<< MOVERS

PAN-EUROPEAN. Finnish molecular biologist Marja Makarow has become the first woman to be named head of the European Science Foundation (ESF), headquartered in Strasbourg, France. Makarow, currently a research administrator at the University of Helsinki, says she wants to build stronger scientific links across Europe by developing pilot programs that encourage cooperative funding and networking, in addition to strengthening existing programs such as the European Collaborative Research scheme. She also wants to see the 33-year-old foundation play a bigger role in the policy arena by engaging researchers from different disciplines, including the social sciences.

"There are opportunities to learn from each other," she says of ESF's 75 member organizations from 30 countries. "Our great challenge is that money does not cross borders. … The vast majority of research money lies with national agencies." Makarow will succeed outgoing chief John Marks in January 2008.

Selecting flower color 376

Early human settlement 377

GLOBAL WARMING

Nobel Peace Prize Won by Host Of Scientists and One Crusader

The announcement came as a shock to Robert Watson. "It would never have crossed my mind that a scientific assessment process would be named in a Nobel Peace Prize," he says. "If anyone had told me that could happen, I would have said, 'You have to be smoking something.' " But stone-cold sober the Norwegian Nobel Committee was when it awarded the prize to the United Nations–sponsored Intergovernmental Panel on Climate Change (IPCC)—which Watson chaired from 1997 to 2002—and to Al Gore for their "efforts to build up and disseminate greater knowledge about man-made climate change" because such change may increase "the danger of violent conflicts and wars, within and between states."

Winners all. IPCC chair Rajendra Kumar Pachauri (*left*), representing several thousand scientists, and Al Gore share the Nobel Peace Prize for creating and spreading knowledge of climate change.

The odd-couple winners are a good match, most scientists believe. On the one hand, there's the organization of thousands of unpaid, nearly anonymous researchers meticulously assessing the state of climate science; on the other, a former politician using that science to underpin his media-savvy campaign to save the world from climate catastrophe. "The combination of IPCC, with its very careful examination of scientific knowledge, and Al Gore's ability to bring the message to politicians and the public" has worked well, says Bert Bolin, the first chair of IPCC. Not that their work is done. There's still the matter of steeling the public's will to meet the costs of countering the threat.

On the IPCC side, the winners are legion. "This is an honor that goes to all the scientists and authors who have contributed to the work of the IPCC," says Indian engineer and economist Rajendra Kumar Pachauri, current IPCC chair. The award recognizes a vast amount of unpaid hard work on their part, says geoscientist Michael Oppenheimer of Princeton University, who has served IPCC in various capacities since the United Nations established the body in 1988. "There's an incredible amount of time involved," he says, flying to meetings in every corner of the world, hammering out consensus, responding to thousands of reviews, and extracting government approval word by word for three different working groups for each report (*Science*, 9 February, p. 754). "There is a price," says Oppenheimer. "People burn out."

Working against burnout is "a sense of community responsibility," says Oppenheimer. "A free society provides the space so you can do science" and create knowledge. In return, he says, climate researchers serve on IPCC to distill that knowledge in a credible way for policymakers. Adds Watson: "They want informed political decisions. If they want their science to be part of informed policymaking, the IPCC is the vehicle." And then there is self-interest. "I get more out of IPCC than I put in," says Oppenheimer. "IPCC meetings are very useful." They force a critical analysis of a scientist's own specialty and provide exposure to the top people in other fields, scientists say.

The other winner of the prize is far more familiar to the public. But Gore has also been well-known to the scientific community for decades. Scientists say few politicians have relied upon or involved more researchers in their policy work than Gore. "My relationship with Al Gore was born in combat," says climate researcher Stephen Schneider of Stanford University in Palo Alto, California, who recalls a 1981 hearing then-representative Gore held in which Schneider opposed a move by the Reagan Administration to cut climate research. "We were soldiers in the same war … for 25 years."

Climate researchers have known Gore as the rare policymaker who brings scientists in—and listens. When he visited Lamont-Doherty Earth Observatory in Palisades, New York, as a senator, recalls geochemist Wallace Broecker, "he said, 'I don't want a tour. I just want to sit around a table with some of your climate people.' " While Gore was writing his 1992 book *Earth in the Balance*, recalls atmospheric chemist Michael McElroy of Harvard University, the then-senator spent 2 hours on the phone nailing down a "pretty subtle chemical point" about ocean acidification. "He came into these issues with a visceral feel that this was an important issue," says McElroy, "like the Vietnam War had been when he was a young man."

Schneider thinks the award to both Gore and IPCC recognizes their dual roles in promoting climate science. "We provide the credibility the Gores and Blairs and Schwarzeneggers need," he says of the panel. And Gore's treatment of that science? "He did a pretty good job of communicating complex scientific information to a lay audience," says McElroy of Gore's film *An Inconvenient* ▶

CREDITS (LEFT TO RIGHT): TANUSHREE PUNWANI/REUTERS/LANDOV; AP

Scientific siting sagas
380

Origins of seafaring
388

Truth. "If it was a scientist doing it, it would be different. But I don't think there were any glaring errors." The publicity, Broecker says, accomplished far more than IPCC's scientists could have done on their own: "Gore put it in a way that people listened. We're much further along to meaningful action [to cut emissions] because of him."

IPCC led the way, Watson says. Its reports forging increasingly strong links between human activity and global warming were instrumental in moving nations toward drafting and signing the Kyoto Protocol for cutting greenhouse gas emissions, he says. But more recently, says Oppenheimer, other forces have come into play: high oil prices and a new energy crisis; events ascribable to global warming, such as the dwindling of Arctic sea ice; and weather events such as Hurricane Katrina that are at least analogs of weather in a greenhouse world.

And then "along comes Al Gore," says Oppenheimer. The end result has been an explosion of media attention and, in the United States, unprecedented political debate and even emission-cutting legislation. But it's not over, warns political communications researcher Matthew Nisbet of American University in Washington, D.C. IPCC and Gore may have raised awareness broadly and stoked concern among the already environmentally attentive, but by Nisbet's reading of the polls, the broad support for emissions cuts that will hurt is nowhere near there. Activists, he says, need a new message.

–RICHARD A. KERR AND ELI KINTISCH

With reporting by Pallava Bagla.

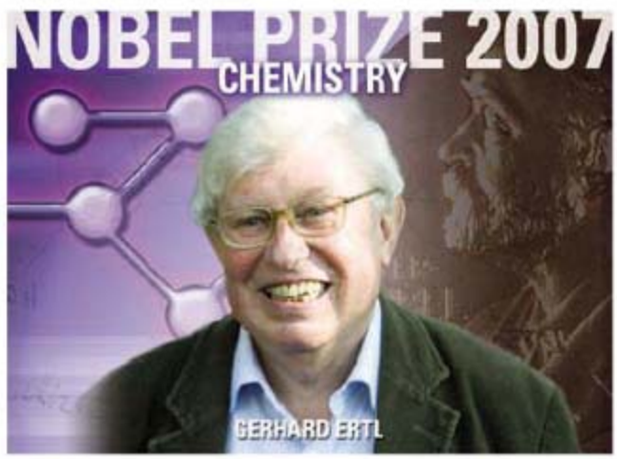
NOBEL PRIZES

Chemistry Laureate Pioneered New School of Thought

Now that's a birthday present! Instead of receiving the random necktie on his 71st birthday last week, Gerhard Ertl was awarded this year's Nobel Prize in chemistry. Ertl, a physical chemist at the Fritz Haber Institute of the Max Planck Society in Berlin, Germany, won for developing methods that reveal how chemical reactions take place on metals and other surfaces. Those techniques have led to results as diverse as new catalysts that remove poisonous carbon monoxide from car exhaust and an understanding of how stratospheric ice crystals supercharge chlorine's ability to destroy the planet's protective ozone layer.

"This is really well deserved," says Ralph Nuzzo, a surface chemist at the University of Illinois, Urbana-Champaign. "Ertl is a titan." John Vickerman, a chemist at the University of Manchester in the U.K., agrees. "The reactions occurring at surfaces are very difficult to probe because there are so few molecules involved, and they frequently occur very rapidly," he says. "Furthermore, the scientist has to distinguish what is happening in a layer one molecule thick from the rest of the solid. Ertl developed very sophisticated physical tools to identify the chemistry occurring at the surface." The Royal Swedish Academy of Sciences, which awards the Nobel Prizes, says Ertl was selected not for developing a particular tool, technique, or discovery, as is often the case, but because "he established an experimental school of thought for the entire discipline."

One early example was in figuring out how iron-based catalysts convert hydrogen and nitrogen into ammonia, a critical industrial process for making fertilizers. This conversion, known as the Haber-Bosch process, combines dinitrogen molecules from the air with dihydrogen molecules. Earlier studies had revealed that the slowest step in the process was one in which nitrogen molecules adsorb onto iron particles in a manner that primes them for combining with hydrogen. Researchers didn't know whether the tightly bonded nitrogen molecules reacted with hydrogen intact or whether they broke apart first. Using spectroscopic techniques and other tools, Ertl revealed the complete seven-step process whereby nitrogen and hydrogen molecules land on an iron surface, break apart, and react to form ammonia.



Many happy returns. After Gerhard Ertl won the Nobel on his birthday, colleagues toasted him with champagne and German pretzels.

CREDIT: TOBIAS SCHWARZ/REUTERS/LANDOV; JUPITER IMAGES

After receiving the announcement last Wednesday, about 200 of Ertl's colleagues toasted him with champagne and German pretzels on the shaded lawn of the Fritz Haber Institute. After Ertl fielded a few questions from TV reporters, the crowd broke out in a rousing round of "Happy Birthday to You" (in English).

In an earlier phone interview with *Science*, Ertl was quick to offer credit to fellow researchers. His field, he says, was propelled by the parallel development of many surface characterization techniques. And, he adds, many scientists were adept at applying them—including Gabor Somorjai of the University of California, Berkeley, with whom he shared the 1998 Wolf Prize in Chemistry for their work in surface science. "I was a little bit disappointed he didn't share [the Nobel Prize] with me," Ertl says. Last week, several chemistry bloggers went further, arguing that Somorjai deserved recognition for his vital role in laying the foundations of surface science.

For his part, Somorjai says simply that he does not understand how award decisions are made. But he notes that in the 1980s, he began steering away from ultrahigh-vacuum surface science to study reactions at solid-liquid interfaces, among other things. By contrast, Somorjai says, "Ertl stayed in there all through his life."

–ROBERT F. SERVICE

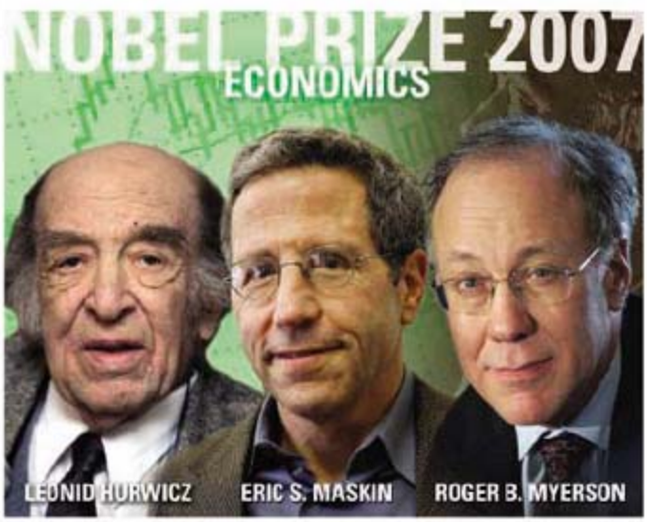
With reporting by Gretchen Vogel in Berlin, Germany.

NOBEL PRIZES

Three Economists Lauded for Theory That Helps the Invisible Hand

Scottish philosopher Adam Smith asserted that when everyone acts out of self-interest, everyone will eventually benefit, as if a benevolent "invisible hand" molds the economy. Economists now know that view is naive: In some situations, rational people will act in ways that leave everybody a loser. But such dreary outcomes can sometimes be avoided, thanks to work that earned three Americans the Nobel Prize in economics.

Leonid Hurwicz of the University of Minnesota, Twin Cities, Eric Maskin of the Institute for Advanced Study in Princeton, New Jersey, and Roger Myerson of the University of Chicago, Illinois, developed "mechanism design theory." The theory aims to find schemes, or "mechanisms," that ensure that acting in self-interest will indeed lead to benefits for all. Today, its applications range from how best to auction broadcast rights and other public resources to contract negotiations and elections.



Everybody wins. Leonid Hurwicz, Eric Maskin, and Roger Myerson (*left to right*) have won the Nobel Prize in economics.

"At first, I thought it was some kind of a joke," says Hurwicz, of hearing of his award. At 90, Hurwicz is the oldest person to win a Nobel. He says colleagues had told him that he might win, "but not in recent years." The prize is well-deserved, others say. "I was riding in the car [and discussing the prize] with somebody yesterday, and these were the three names that came up," says W. Bentley MacLeod, an economist at Columbia University.

Mechanism design theory starts with the recognition that unbridled self-interest doesn't always lead to the greater good. For example, if the people of a town were asked to chip in to build a bridge, each person would benefit by underestimating his or her share and letting others bear the cost. So for lack of funds, the bridge would never get built. That sort of a logically unavoidable lose-lose situation is known as a Nash equilibrium.

In the 1960s, Hurwicz pioneered the study of how to avoid such dead ends by fiddling with the rules of an economic or social interaction so that the most beneficial state and the inevitable equilibrium state are one and the same. "It's a little Machiavellian," says Gabrielle Demange of the Paris School of Economics. "You design a game so that in the end the Nash equilibrium comes out to be what you want." For example, each person could be required to pay what *others* think the bridge is worth, thus eliminating the incentive to lie.

Maskin, 57, and Myerson, 56, expanded on Hurwicz's work. In 1977, Maskin developed a criterion for determining just when it's possible to find rules that will guide self-interested participants to the desired end. Starting in the late 1970s, Myerson showed that whenever a mechanism exists, it is also possible to find one that gives participants an incentive to tell the truth, an insight that makes it much easier to devise practical mechanisms.

Relying heavily on game theory, the laureates' work has been largely abstract and formal. "My methodology is to invent simple little worlds in which there is just a bit that we don't understand and can study," Myerson says. Nevertheless, the theory may play a role in confronting perhaps the most complex and pressing problem facing humanity today, climate change, by helping to set up incentives that encourage consumers and countries to minimize greenhouse gas emissions. "Mechanism design should definitely be pertinent to the problem," Maskin says. "But first we have to decide exactly what we're trying to accomplish."

–ADRIAN CHO

CREDITS (TOP TO BOTTOM): ERIC MILLER/REUTERS/LANDOV; EMILE WAMSTEKER/BLOOMBERG NEWS/LANDOV; TIM BOYLE/BLOOMBERG NEWS/LANDOV; JUPITER IMAGES

Sequestration (in) Rocks

Last week, the U.S. government took two important steps on the long road to testing the feasibility of burying carbon dioxide to combat global warming. The Department of Energy chose three sites in Texas, North Dakota, and Alberta, Canada, to inject 1 million or more tons of CO_2 from coal plants in an effort to sequester carbon emissions from power plants. And the Environmental Protection Agency said that it would begin crafting rules on regulating such large-scale injection projects. The rules will help maintain clean drinking water during massive injection projects.

–ELI KINTISCH

New SETI Array Deployed

Microsoft co-founder Paul Allen threw a switch last week christening an array of 42 antennas designed to search for signals from other intelligent life in the universe. Although the Search for Extraterrestrial Intelligence (SETI) has been going on for more than 3 decades, the Allen Telescope Array will expand the search 1000-fold in the next 20 years and eventually could include 350 antennas at a site 480 kilometers north of San Francisco, California. Allen has pledged $11.5 million for the venture, which Congress forced NASA to abandon in the early 1990s.

–ANDREW LAWLER

Nuclear Deal in Deep Freeze

NEW DELHI, INDIA—The U.S.–India nuclear agreement hit a roadblock last week when India's Communist parties threatened to withdraw their support from the government if the pact went forward. The deal is likely to be consigned to cold storage, politicians say, possibly to be resurrected in 2009 after both countries have held national elections.

The completion of the process leading to the so-called 123 Agreement would have allowed India to purchase equipment and fuel for its civilian nuclear program on the U.S. and world markets, ending 4 decades of isolation following India's explosion of a nuclear device in 1974. Prime Minister Manmohan Singh repeated his support for the plan, calling it an "honorable deal, good for the country, good for the world." But in a tactical climb-down, Singh noted that although it would be a "disappointment" if the deal does not go through, it would not be "the end of life." Reacting to the announcement, M. R. Srinivasan, a member of the Indian Atomic Energy Commission, said, "A delayed deal is better than a bad deal."

–PALLAVA BAGLA

EVOLUTION

Natural Selection, Not Chance, Paints the Desert Landscape

Desert snow, a flower that lives in the Mojave Desert, has a colorful history—literally and figuratively. The five-petaled *Linanthus parryae* comes in purplish-blue and white varieties; it sometimes carpets dusty landscapes in a single color and sometimes in a blue-white mosaic. Sixty years ago, studies of these patterns provided key support for a powerful evolutionary theory. Now, two evolutionary biologists have found that the theory doesn't hold in this species.

At issue is the relative role of randomness in genetic differentiation within a population. Did the chance increase in frequency of a new version of a gene—for example, one that tinted desert snow blue—and the luck of the draw result in the blue blooms flourishing in some places and not others? Such serendipity is called genetic drift, and it contrasts with the idea that fitness in a particular environment—natural selection—not chance, is responsible for the successful spread and distribution of these blue and white flowers.

Researchers began studying *Linanthus* in the early 1940s, most notably systematist Carl Epling and evolutionary biologists Theodosius Dobzhansky and Sewall Wright. Epling and Dobzhansky, and later Wright, attributed the flowers' distribution to genetic drift: Blue flower seeds happened to land on the far side of a particular ravine, for example, and spread, isolated from the white ones by the forbidding habitat at the bottom of the ravine.

Epling later decided that natural selection was important, but Wright, based on his continued work with this species, concluded that genetic drift was key. He proposed that the larger a population, the more likely new versions of a particular gene would take hold in a subset of that population, setting the stage for some subsets to head in different evolutionary directions. He called this idea the shifting balance theory. That work has been cited more than 1400 times. Nonetheless, evolutionary biologists have been arguing ever since about how right Wright was.

In 1988, Douglas Schemske of Michigan State University in East Lansing and Paulette Bierzychudek of Lewis & Clark College in Portland, Oregon, decided to weigh in on the controversy. "Because none of these studies had directly estimated natural selection, we thought it was necessary to mount a long-term field project to resolve the dispute," Schemske recalls. That year, they started tracking the distribution and fitness of *Linanthus*.

They reported in 2001 that natural selection could be intense, playing a larger role in shaping the distribution of flower color than Wright realized. Now, in an early online release of *Evolution*, Schemske and Bierzychudek have pinpointed strong environmental differences that likely keep blue flowers to one side of the ravine and white flowers to the other. The work "provides a very nice historical perspective on this key system, one that has crept into a lot of textbooks," notes evolutionary biologist Michael Lynch of Indiana University, Bloomington. "They clearly don't come down on the side of Wright."

Desert blooms. For decades, researchers have debated why proportions of white and blue *Linanthus parryae* (*top*) vary across arid landscapes.

Schemske and Bierzychudek focused on two 500-meter-long swaths along a 25-meter-wide ravine with blue flowers on the west side and white ones on the east. Over 7 years, they counted the blue and white blossoms and noted changes in the distribution of the two colors. They looked at the distribution of allozymes—different versions of a given protein—in flowers on both sides of the ravine. In addition, they planted some white-flower seeds on the west side and blue-flower seeds on the east and vice versa, monitoring seed production in these experimental plots. Because one year was quite wet and another quite dry, the researchers were able to assess the two colored flowers' fitness relative to precipitation. They also analyzed the makeup of the soil and plant communities on both sides of the ravine, finding big differences in both. "It was rigorous fieldwork and careful analysis, work that addresses important questions with exceptional clarity," says plant population biologist Vincent Eckhart of Grinnell College in Iowa.

The sides were more than 95% blue or white. But the distribution of the allozymes did not parallel that of the flower color. Had genetic drift caused the color pattern, the distribution of at least some allozymes should have been skewed as well, Schemske and Bierzychudek note. In the seed-transplant studies, each color flower typically did best on its own turf, indicating that selection played a role. "Our data strongly suggest that it's no accident that there are only blue survivors on the west side and only white survivors on the east side," says Bierzychudek.

Furthermore, the soil and community composition of the two sides of the ravine were different—one side had a much higher proportion of creosote bushes, for example—providing strong evidence of environmental differences that could favor one flower color over another.

"The study shows the unimportance of drift in *Linanthus*," says evolutionary biologist Masatoshi Nei of Pennsylvania State University in State College. "In this sense, [the] finding shakes the ground of the shifting balance theory." But he is cautious about making generalizations, given that other studies suggest otherwise: "The relative importance of selection and drift depends on the genes and populations studied." **–ELIZABETH PENNISI**

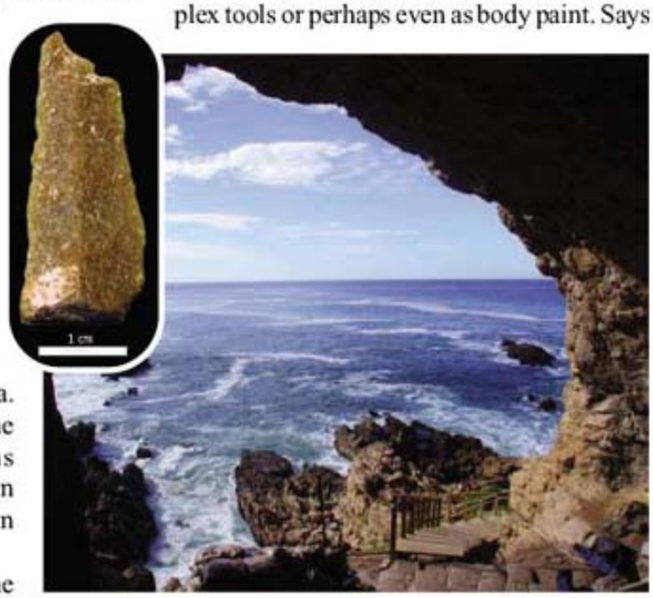
CREDITS: D. W. SCHEMSKE AND P. BIERZYCHUDEK, *EVOLUTION* (ADVANCED ONLINE, 2007); BLACKWELL PUBLISHING INC.

ARCHAEOLOGY

Coastal Artifacts Suggest Early Beginnings for Modern Behavior

Modern humans first appear in the fossil record of Africa between 160,000 and 195,000 years ago, with skulls and bones that are virtually indistinguishable from ours. But looking like us doesn't necessarily mean that they acted like us. Indeed, researchers have debated intensely about when *Homo sapiens* began to act sapient by producing complex tools and manipulating symbols.

Now, an international team of researchers says that some key elements of modern behavior were in place by 164,000 years ago, pushing back the appearance of some of these activities by 25,000 to 40,000 years. The team found complex stone bladelets and ground red pigment—advances usually seen as hallmarks of modern behavior—coupled with the shells of mussels, abalone, and other invertebrates in a cave in South Africa. These ancient clambakes are the earliest evidence of humans including marine resources in their diet, according to a report in this week's issue of *Nature*.



Room with a view. Early *Homo sapiens* ate shellfish and worked with ochre and stone tools (*inset*) in this South African cave.

Not everyone agrees that the artifacts add up to a major cognitive shift. But to paleoanthropologists such as Sally McBrearty of the University of Connecticut, Storrs, the package provides "strong evidence" that these people were manipulating symbols. That "supports the gradual rather than sudden or rapid accumulation of more complex behaviors," adds Alison Brooks of George Washington University in Washington, D.C.

The team found the shells, tools, and pieces of red ochre cemented in the wall of a cave at Pinnacle Point on the Cape of South Africa, on the coast of the Indian Ocean. Using uranium series and optically stimulated luminescence dating, the team dated the sediments to about 164,000 years, during a glacial period that left Africa cool and dry. These humans might have started to eat marine resources as a "famine food" because of a harsh environment, says team leader Curtis Marean of Arizona State University's Institute of Human Origins in Tempe.

Although the team found no human bones, the ancient people did leave behind a trail of stone flakes that the team identifies as bladelets, small points used by more recent humans as advanced projectile points. If so, this would push back the appearance of true bladelets by at least 90,000 years. Other researchers caution, however, that the points may have been made by accident rather than on purpose. The pieces of red ochre were worn down, suggesting that these people were using ochre paste as glue to make complex tools or perhaps even as body paint. Says Marean: "You put that dietary, technological, and cultural package together, and all of a sudden it looks like archaeological sites from 2000 years ago."

But using "little bits of red ochre" pales in comparison with the advances that appear 50,000 years ago in Europe, when humans began to draw animals, shape beads, and bury their dead in elaborate graves—changes that enhanced reproduction and are linked to dramatic population expansions, says paleoanthropologist Richard Klein of Stanford University in Palo Alto, California. By themselves, the Pinnacle Point artifacts would not confer such a significant reproductive advantage, says Klein.

Marean, however, thinks the behavioral changes were so important that they might have been one of the catalysts for the birth of our species. He is searching even older sediments to pinpoint when these behaviors emerged.

–ANN GIBBONS

CREDITS: COURTESY OF THE MOSSEL BAY ARCHAEOLOGY PROJECT

Remains Remain Controversial

Jockeying over what constitutes a native American may resume after the Senate Indian Affairs committee approved a bill (S. 2087) late last month that would redefine the term under the Native American Graves Protection and Repatriation Act. Pro-research groups say the change could prevent scientists from studying ancient remains, whereas Indian groups say it would merely clarify the law's original intent.

Tribal activists have been trying to reverse a federal court ruling in 2004 that said the law did not apply to the 9000-year-old bones of the culturally unidentified Kennewick Man, clearing them for scientific study. S. 2087, a collection of technical amendments to Indian law, adds two words to the definition of "Native American" to make it cover any member of a tribe or culture that is "or was" indigenous to the United States. With a crowded fall calendar, no Senate floor vote is expected in the near future. Representative Doc Hastings (R–WA) is expected to reintroduce a measure in the House shortly that would counter the proposed change.

–CONSTANCE HOLDEN

NSF Shortens Drilling Season

A funding crunch is forcing the National Science Foundation (NSF) to shorten by 4 months annual deep-sea drilling operations beginning in 2009, according to Steven Bohlen of the Joint Oceanographic Institutions (JOI), the NSF-funded operator of the U.S. drill ship *JOIDES Resolution*. "Our operating costs are well beyond what we anticipated," he says, due to the escalating costs of ship fuel, drilling gear, and maintenance. Add in NSF's commitments to support ocean-observing systems and non–drilling-ship operations, and "there are not sufficient funds to support the drill ship for science for the entire year," says Bohlen. JOI will be pursuing work with petroleum companies and other science agencies that Bohlen hopes will fill in the looming gaps. "We're definitely scrambling here. We're worried," he says.

"Is it a big deal?" says Terry Schaff, director of government relations with Woods Hole Oceanographic Institution in Massachusetts. "It would be good if there was enough funds to run it for a whole year," he says. But most ships that run U.S. academic oceanographic research run between 250 and 300 days a year, he points out. "Most of the ships haven't run a full year for a while. It's not a terribly unusual situation."

–RICHARD A. KERR

ASTRONOMY

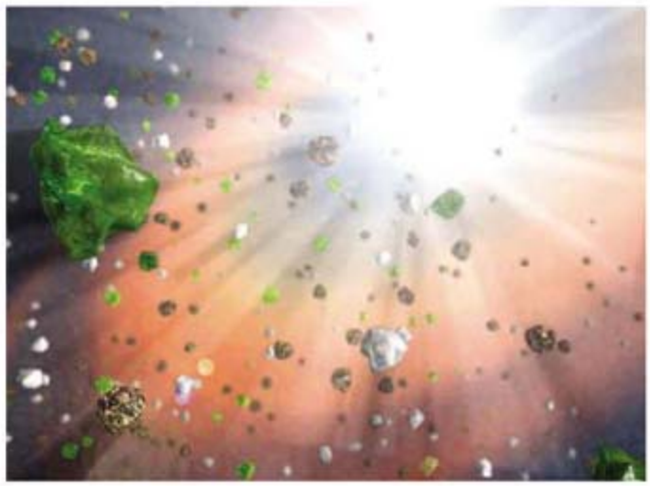
Space Sighting Suggests Stardust Doesn't Have to Come From Stars

Microscopic rubies and sapphires arise in black hole winds. Using NASA's Spitzer Space Telescope, astronomers spotted the telltale spectroscopic fingerprints of these unpolished microgems in space near a supermassive black hole. Many other dust species also showed up, including crystalline minerals that make up sand, glass, and marble. Team leader Ciska Markwick-Kemper of the University of Manchester, U.K., says the find may help explain the abundance of dust particles in the very early universe.

"It's a spectacular find," says astrochemist Rens Waters of the University of Amsterdam in the Netherlands. "If pressures and temperatures in supermassive black hole winds are favorable for dust production, huge quantities of dust could be produced in this way."

The universe started out with a mixture of hydrogen and helium, the two lightest elements. Heavier elements such as carbon, oxygen, silicon, and magnesium formed by nuclear fusion in the first generation of extremely massive stars. Supernova explosions then dispersed these heavy elements through space, where some of them condensed into dust particles—the building blocks of planets such as Earth. However, many components of dust form only in the calm outflows of dying sunlike stars. So astronomers have been baffled to observe healthy amounts of dust at a time in the universe's history when sunlike stars were still in their infancy.

In 2002, astrophysicist Martin Elvis of the Harvard-Smithsonian Center for Astrophysics in Cambridge, Massachusetts, suggested that dust could form in the winds of supermassive black holes that sit in the cores of young galaxies, sucking in matter with their enormous gravity. These gluttonous monsters are "messy eaters," says Sarah Gallagher of the University of California, Los Angeles, spilling and blowing much of their food into space, including heavy elements from supernovae. The balmy temperatures and high densities in these winds could forge dust particles, including crystalline silicates and tiny rubies, from these elements, Elvis theorized.



Matter maker? Perishable compounds near the heart of a galaxy hint that the universe has more than one way of cooking up cosmic dust.

CREDIT: NASA/JPL-CALTECH/T. PYLE (SSC)

Now, analysis of light from a supermassive black hole in a galaxy some 8 billion light-years away supports Elvis's idea. In the Spitzer observations, Markwick-Kemper, Gallagher, and their colleagues detected many mineral species previously seen only in the outflows of dying sunlike stars, such as forsterite (Mg_2SiO_4), periclase (MgO), and corundum (Al_2O_3), the mineral that constitutes ruby and sapphire. Because many of those minerals are easily destroyed by energetic radiation from stars or by interstellar shock waves, the observations suggest that the dust has been freshly formed in the black hole winds.

The case isn't closed. In their paper in the 20 October issue of *The Astrophysical Journal Letters*, Markwick-Kemper and her colleagues note that part of the early universe's dust could still have come from supernova ejecta. Says Waters: "The origin of dust is still shrouded in lots of mysteries." **–GOVERT SCHILLING**

Govert Schilling is an astronomy writer in Amersfoort, the Netherlands.

SCIENCE FUNDING

U.K. Spells Out Boost in Medical Research

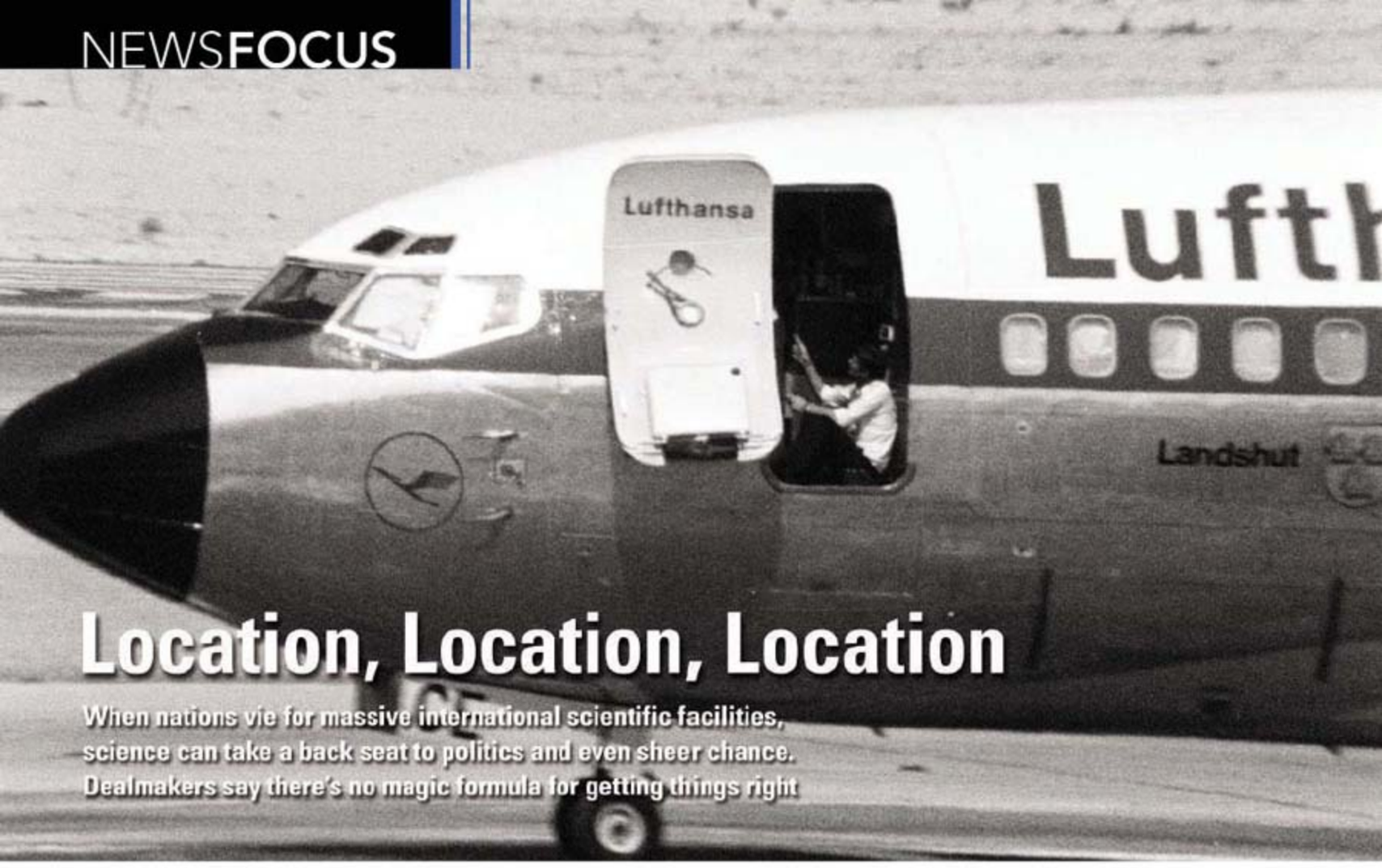
In 10 years as the U.K. government's finance chief, Gordon Brown engineered substantial and steady growth in research funding. Now, as prime minister, Brown is continuing that trend. Last week, the government's Comprehensive Spending Review (CSR)—a statement of spending plans issued every 2 or 3 years—signaled a boost of £300 million (about $600 million), to £1.7 billion, in medical and health research over the next 3 years. "This is nothing less than good news," says Hilary Leevers, acting head of the Campaign for Science and Engineering in the U.K.

The government had previously announced that it intended to boost the overall level of funding for science and university research from £5.4 billion to £6.3 billion over the same 2008–11 period. CSR reveals how that increase will be divvied up. Around half goes to the U.K.'s seven research councils, which distribute grants to scientists at universities and national labs. They will see their £2.8 billion annual funding boosted on average by 5.4%.

The emphasis on medical and health research continues a process begun earlier. In 2006, Brown appointed David Cooksey, a venture capitalist who has advised the government on medical research, to figure out the best way of combining all the government's medical and health research spending into a single fund. Last December, acting on Cooksey's recommendations, Brown created the Office for Strategic Coordination of Health Research (OSCHR).

OSCHR oversees the activities of the Medical Research Council (MRC) and the Department of Health's National Institute for Health Research to promote a new emphasis on "translational" research—taking basic science results and turning them into usable drugs or treatments. CSR—which does not need parliamentary approval—boosts the combined budgets of these two bodies by £300 million. "There's been a need for an increase for some time, and a need for a better connection between the MRC and the Department of Health," says Michael Rutter, clinical vice president of the Academy of Medical Sciences, although he expressed concern that the emphasis on translational research "doesn't lead to a reduction in funding for basic science."

Leevers has similar concerns. The research councils have recently begun requiring information about the economic impact of research on grant applications, a change that some researchers worry would put basic research proposals at a disadvantage. "The government ardently believes in the drive toward innovation," she says. "But you have to have the bedrock on which to innovate." **–DANIEL CLERY**



Location, Location, Location

When nations vie for massive international scientific facilities, science can take a back seat to politics and even sheer chance. Dealmakers say there's no magic formula for getting things right

ON THE NIGHT OF 17/18 OCTOBER 1977, a Lufthansa airliner sat on the tarmac of Mogadishu airport in Somalia and the world held its breath. Four days earlier, terrorists from the Popular Front for the Liberation of Palestine had hijacked the Boeing 737 en route from Majorca to Frankfurt and demanded $15 million and the release of 11 members of an allied terrorist group, the Red Army Faction (RAF), who were in prison in Germany. Over the following days, the plane landed in Rome, Larnaca, Bahrain, Dubai, and Aden before coming to a stop in Mogadishu, where the hijackers dumped the body of the pilot—whom they had shot—out of the plane. They set a deadline that night for their demands to be met.

At 2 a.m. local time, a team of German special forces, the GSG 9, which had been tailing the plane across the Mediterranean and Middle East, stormed aboard. In the fight that followed, three of the four terrorists were killed and one was captured with bullet wounds. All the passengers were rescued uninjured. Far from the action, the resolution of the hijacking had a surprising side effect: the Joint European Torus (JET), an experimental nuclear fusion reactor being planned by European nations, ended up being built in the United Kingdom rather than in Germany.

On the day the hijacking ended, British prime minister James Callaghan arrived in Bonn for a summit meeting and was met by German chancellor Helmut Schmidt with the words: "Thank you so much for all you have done." The reason for his gratitude was that Britain's Special Air Service (SAS), the Army's special forces unit, had advised the GSG 9 and provided them with specially designed stun grenades, which the German commandos used to incapacitate the hijackers during the storming of the plane.

Golden age. CERN's dedication in 1955 made the lab a model for big international projects.

Because of this help, Schmidt settled an issue that had recently divided the two countries: where to build JET. Most of the nine members of what was then the European Economic Community (EEC) supported Culham near Oxford, but Germany was holding out for Garching, home of its own fusion research lab. At a cabinet meeting the day after meeting Callaghan, Schmidt backed Culham, and on 25 October, the site was approved by EEC research ministers.

It's not often that acts of terrorism play a part in international research collaborations, but there comes a time in the development of many such projects—usually around the issue of choosing a site—when national pride and cross-border rivalries can take over from technical considerations. In such situations, the scientists who have carefully nurtured a project for years become bit players as international power politics is played out.

When politicians stumble, the process can become so divisive that it threatens the whole project and international relations as well. Such was the case with ITER, a global fusion research project that is the successor to JET. In late 2003, ITER's site-selection process descended into 18 months of mudslinging and frantic shuttle diplomacy. Although an

CREDITS (TOP TO BOTTOM): AP; CERN

Payback. Help in storming hijacked Lufthansa flight 181 got Britain an experimental fusion reactor.

amicable resolution was finally achieved, there were moments when the project's future looked in doubt, and many consider the episode a low-water mark in international scientific collaboration. "I haven't talked with anyone who was happy about the ITER process, even those who won," says an international official who asked not to be named.

So is there a better way to choose the site for an international facility? Those projects currently on the drawing board—including the next multibillion-dollar particle physics machine, the International Linear Collider (ILC)—don't seem to have agreed on the best method, but with the scars of ITER still raw, they are treading very carefully.

Physicists with a mission

The model for international collaborations, most agree, is CERN, Europe's particle physics lab. Soon after the Second World War, a group of prominent physicists, including Pierre Auger, Isidor Rabi, Eduardo Amaldi, and Lew Kowarski, bullied, coaxed, and cajoled European governments and the continent's physicists into supporting an international particle physics lab. The aim was both to rebuild European science and to foster international cooperation. In February 1952, 11 nations signed up to the provisional CERN and soon four sites were under consideration: Geneva, Copenhagen, Paris, and Arnhem in the Netherlands.

A site-selection committee began visiting the sites prior to a meeting of the provisional CERN council in October 1952. By this time, Paris had slipped in the rankings because it was considered too big, too expensive, and plagued by labor strikes. Copenhagen was strongly opposed by the French. Geneva made a strong case as an international city: home of the defunct League of Nations, and with good tax and customs terms. Reportedly, on the day the selection committee visited Arnhem, it was pouring with rain. The panel found a town with only two hotels, no university, no international school, and only a few foreign newspapers at the train station newsstand. At the council meeting in October, the delegations lined up behind Geneva.

The next hurdle was Swiss public opinion. Eastern bloc countries had declined to join the project, and communist politicians in Switzerland exploited the resulting Western bias. They claimed that the lab would become part of the U.S. atomic system, controlled by bomb manufacturers. A heated debate in the Geneva state council spilled out into fistfights in the corridors. Voters in the Canton of Geneva, fearing the health effects of radiation and a threat to Swiss neutrality, petitioned for a referendum on CERN, to be held on 29 June 1953. In the run-up, physicists made a hectic round of speeches and rallies—the city was abuzz with scientific debates. On the day, only 7332 voted against the lab—fewer than had signed the original petition—and 16,539 voted in favor. On 1 July 1953, the provisional council voted CERN into existence.

Undone deal. The European Synchrotron Radiation Facility wound up in Grenoble after last-minute political maneuvering changed the site from Strasbourg.

CREDIT: ESRF

The center soon became a model for other cross-border collaborations: the Institut Laue-Langevin (ILL, a neutron source), the European Molecular Biology Laboratory (EMBL), the European Space Agency (ESA), and the European Southern Observatory (ESO). Relatively few sparks flew in the discussions over siting these organizations. ESA is headquartered in Paris, but has other facilities in all its major funding countries apart from the United Kingdom. ESO has its base in Garching, Germany, but its telescopes are all in Chile. "Everyone is happiest when the [location] issue doesn't come up," says the international official, such as when the best site is not in one of the funding countries.

But such harmony grew increasingly difficult to maintain as politicians became increasingly interested in scientific facilities for the international prestige they brought and the money they injected into local economies. In the mid-1970s, European researchers identified the need for a large synchrotron radiation source, a provider of intense laserlike x-rays for physicists, materials scientists, and molecular biologists. By the early 1980s, many countries had expressed interest in hosting the machine but it boiled down to horse-trading between the main backers, France and Germany. According to CERN physicist Horst Wenninger, President François Mitterrand and Chancellor Helmut Kohl decided the issue over a breakfast cup of coffee: The site for the European Synchrotron Radiation Facility (ESRF) would be Strasbourg on the French-German border.

But in 1984, researchers and politicians in the French city of Grenoble began agitating for a rethink. According to current ESRF director Bill Stirling, the then ILL director Brian Fender had suggested a vacant site next door to his facility to build on synergies and common services. Grenoble is also home to a number of French national research centers, and prominent scientists lobbied Mitterrand and other politicians. With elections looming, Mitterrand struck a new deal with the Germans. "They were furious in Strasbourg," Stirling says. But ESRF's troubles weren't over. The geology of the site was not ideal, and it was surrounded by vibration-causing roads and rivers. After errors in construction, the concrete slabs supporting the beam lines had to be relaid. But after its difficult birth, the world's first third-generation synchrotron was a great success.

Since ESRF, the movement to build large pan-European labs has faded. These days, it is more common for governments to beef up an existing national lab with new facilities and recruit international partners to help shoulder the burden. Germany is currently starting construction on two such examples: the XFEL x-ray laser at its DESY particle physics lab near Hamburg and the Facility for Antiproton and Ion Research (FAIR) at the GSI heavy ion research lab at Darmstadt.

The bigger they come ...

In recent years, scientists' ambitions have increasingly taken on a global scale, and as the

budgets get bigger, the stakes get higher. The most ambitious project, and the one that really tested the powers of diplomacy was ITER, an experiment designed to prove fusion is a viable source of power for humankind (*Science*, 13 October 2006, p. 238).

The ITER project was started in the mid-1980s. After a global design effort, a redesign, the departure of some members, and the arrival of others, the delegations from six partners—China, the European Union (E.U.), Japan, Russia, South Korea, and the United States—gathered in Washington, D.C., in December 2003 to choose between two candidate sites and sign the agreement that would set the construction ball rolling, at a total cost of some $12 billion. "The higher the stakes, the more difficult the decision is," says Achilleas Mitsos, the E.U.'s former director general of research.

The political atmosphere at the Washington meeting could not have been worse. The E.U.'s proposed site was at Cadarache in southern France, and relations between France and the United States were subzero following France's opposition to the Iraq War, which had begun earlier that year. According to Mitsos, who was the E.U.'s chief negotiator, the United States was determined to get a result in Washington and was unambiguously in favor of Japan's proposed site, Rokkasho. "Clearly, the game was not going to be easy," Mitsos says.

Despite enormous pressure, the E.U. delegation played the long game and convinced the other partners that further technical studies of the two sites were needed. Those studies still failed to signal a clear winner, although European researchers asserted that Rokkasho's position in northern Japan had too high a risk of earthquakes, whereas the Japanese charged that Cadarache was too far from the coast and it would be impossible to move large components that far by road. Japan upped the stakes by offering to pay not the required 40% host contribution but 50%. The E.U., after much handwringing, followed suit.

The E.U. negotiators realized that in order to win they had to come up with a face-saving formula for the loser. The E.U. opened direct discussions with Japan on a set of extra fusion-science facilities to be built in whichever country did not get the main reactor. Negotiations over this "broader approach to fusion" continued in a theoretical fashion through the second half of 2004 and into 2005—Mitsos says he traveled to Tokyo twice a month while other officials shuttled between other capitals. "Russia and China every day became more pro-Cadarache, and the U.S. and Korea every day became less insistent on Rokkasho," he says. Finally, in June 2005, Japan agreed to back Cadarache. "The broader approach was the deciding factor. It allowed Japan to not come out as the loser," Mitsos says.

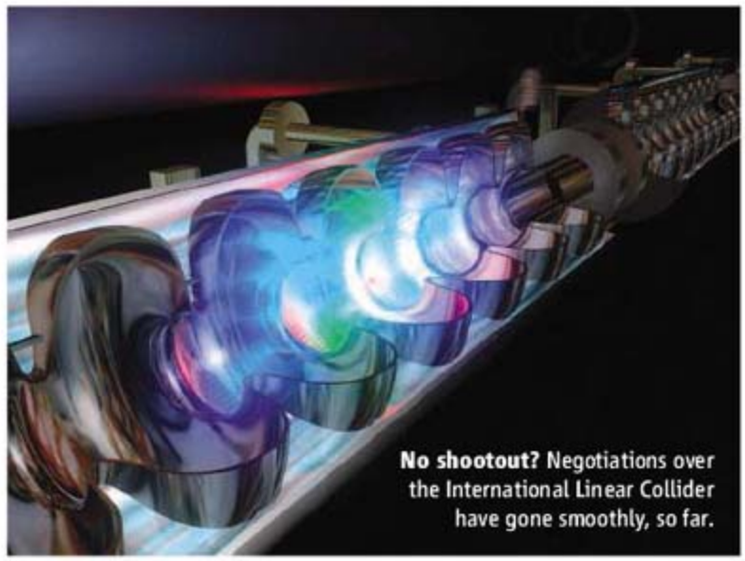
How will the next megaproject avoid the pitfalls that ITER stumbled on? "We're trying hard not to duplicate ITER," says Barry Barish, head of the global design effort for the ILC project, but "if there's a process, I don't know what it is."

The ILC is the next big machine on particle physicists' shopping list. Researchers around the world are currently working on a detailed design for the machine and they've done some testing of "sample sites" in the United States, Europe, and Japan. "We're very early in the process, but probably our biggest lesson from ITER is to avoid the 'all or nothing' situation," Barish says. Although the machine has to be in one place, its high-tech components will be designed, built, and tested at sites across the globe, and it will be managed and governed as a global facility.

Drawing another lesson from ITER, ILC's funders have become actively involved in the planning, even at this early stage. Ian Halliday, former head of the U.K.'s Particle Physics and Astronomy Research Council, helped set up Funding Agencies for the Linear Collider (FALC), which, he says, will allow interested parties to "talk about what everyone wants, identify problems early on, and learn how everyone's funding works." FALC has already acted to smooth out tensions over issues, such as whether to use superconducting magnets in the accelerator or conventional technology, and who should lead the design effort. "It's a gradual process. We might end up without a shootout, but it's in the lap of the gods," he says.

Experts in such international negotiations dismiss the idea that there is some magic formula for resolving disputes. "There isn't such a thing," says Stefan Michalowski, executive secretary of the Organisation for Economic Cooperation and Development's Global Science Forum, a talking shop for senior scientists and science administrators. "Don't try to create general principles," he says, but at a certain stage in a project's planning, "get everyone to agree on the rules." He cites the case of the International Neuroinformatics Coordinating Facility (INCF), a small collaboration for which he was asked to head the site selection committee. All 15 member countries agreed on the criteria for selection beforehand. His committee worked through the process and made its recommendation. "Not everyone was happy, but no bones were broken and the losers got over it."

Although there may not be a magic formula, some sort of oversight authority could play a role. "The only thing that will make a difference is a substantial, European-level central fund for facilities," says Peter Tindemans, spokesperson for the European Spallation Source, a neutron source that has been on the drawing board for more than a decade and will soon be choosing a site. E.U. officials have been thinking along similar lines. When they proposed plans for the latest tranche of the multiyear Framework research program, it contained funds to pay for as much as 20% of the construction cost of pan-European projects. E.U. officials "could participate to provide a package deal, come up with a plan to link projects, and allow everyone to have a stake," says Mitsos. He adds that they even drew up a table, laying out details of funding and where each future facility would go so that all countries got a fair division of spoils.



No shootout? Negotiations over the International Linear Collider have gone smoothly, so far.

CREDIT: DESY

In the budget negotiations last year for the seventh Framework, the funds for infrastructure were slashed and the program can now only help out with the preparatory stages of projects. But Mitsos believes that, in Europe at least, the E.U. will eventually take on the role of dealmaker and guardian of fairness in international projects. "The possibility to draw such a table exists. I'd be surprised if we didn't try again." As for global facilities, they'll have to continue to make up the rules as they go along. **–DANIEL CLERY**

ALZHEIMER'S DISEASE

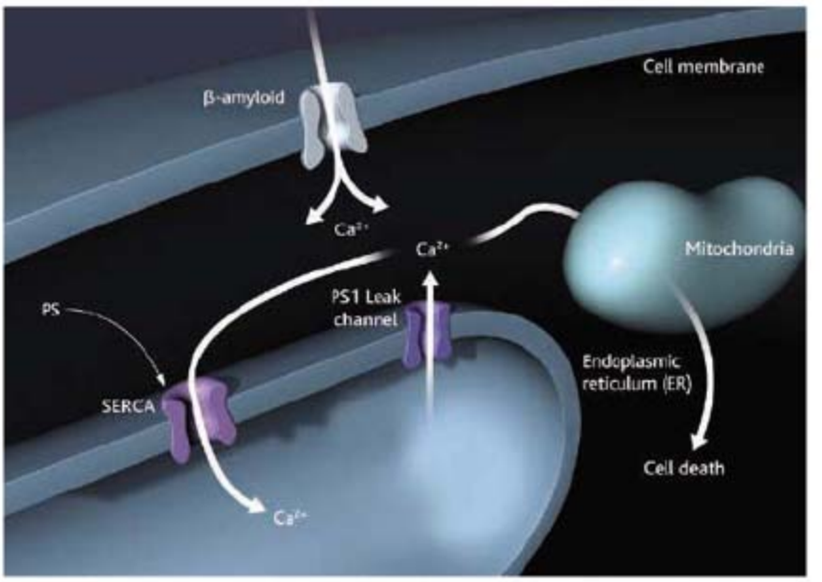
Fresh Evidence Points to An Old Suspect: Calcium

Proteins known to contribute to Alzheimer's pathology have been linked to disturbances in calcium ion regulation that could underlie neuronal death in the disease

Imagine that police discover hundreds of dead bodies over the course of a year and the same suspicious-looking man is standing near each one. A strong circumstantial case for murder, of course. But given that the exact cause of death is uncertain in each case and that no one witnessed the suspect with any obvious weapon, prosecutors would still have a hard time convicting him.

That's essentially the circumstance facing Alzheimer's disease researchers. For years, they've thought that the protein β-amyloid causes the neurodegeneration underlying the fatal illness, but they remain unsure about how it kills brain cells. Now, the mystery may be beginning to unravel.

New evidence supports an old, but somewhat neglected, idea: that β-amyloid, perhaps by forming channels in neuronal membranes, slays brain cells by making them unable to regulate their internal concentrations of ions, particularly calcium ions. Such changes can be "ominous," says Charles Glabe of the University of California, Irvine (UCI). "You just can't go around punching holes in membranes" without endangering the neuron.



Calcium ion portals. Presenilins regulate calcium ion release by the ER into the cytoplasm whereas β-amyloid may form channels that allow the ions in from the cell exterior.

But β-amyloid is only part of the emerging picture. Two additional suspects, known as presenilin 1 and presenilin 2 (PS1 and PS2), have also been linked to Alzheimer's pathology because mutations in their genes can cause the disease. Evidence now indicates that these proteins, too, normally help maintain calcium ion concentrations in neurons and that the disease-causing mutations disrupt this function.

If so, this would be a new role for the presenilins, which were previously shown to contribute to Alzheimer's pathology by clipping β-amyloid out of a larger precursor protein called APP. But if a calcium imbalance does in fact cause neuron death in the disease, a new therapeutic strategy may be possible. "You might block calcium flux as a way of preventing neurodegeneration," says Sam Gandy, an Alzheimer's researcher at the Mount Sinai Medical Center in New York City.

Calcium overload

The idea that calcium overload might be the final insult that finishes off brain neurons in Alzheimer's emerged in the mid-1980s, mainly from a hypothesis put forward by Zaven Khachaturian, then director of the Alzheimer's program at the National Institute on Aging (NIA) in Bethesda, Maryland. Khachaturian, who now heads up the Lou Ruvo Brain Institute and Keep Memory Alive in Las Vegas, Nevada, says that he wanted researchers to focus more on finding the underlying mechanisms of neurodegeneration rather than just describing the brain pathology.

At about the same time, however, much of the Alzheimer's field began concentrating on β-amyloid as the likely nerve cell killer—in part because it's found in the abnormal plaques that stud the brains of Alzheimer's patients. Even more convincing evidence came when researchers found that mutations in APP cause an early onset form of the disease.

Then in the early 1990s, Nelson Arispe of the Uniformed Services University of the Health Sciences in Bethesda, Maryland, and his colleagues provided a possible link between β-amyloid and the calcium hypothesis. When they exposed artificial membranes designed to resemble the cell membrane to β-amyloid, the protein formed channels in the membrane. "Those channels were very particular," Arispe says. "They only permitted the flow of cations [positively charged ions]," such as calcium, into the cell. That fits with numerous observations over the years that exposing nerve cells in culture to β-amyloid causes an increase in their internal calcium ion concentrations.

More recently, Arispe and his Uniformed Services University colleague Olga Simakova provided further support for the idea that calcium disturbances underlie β-amyloid's toxic effects. They found that application of β-amyloid to nerve cells maintained in lab cultures produced an immediate rise in intracellular calcium concentrations followed by the death of the cells. Both effects, they reported in the 9 May 2006 issue of *Biochemistry*, could be inhibited by a peptide they designed to block β-amyloid calcium channels.

Arispe isn't alone in reporting that β-amyloid seems to form ion channels. In 2005, Jorge Ghiso of New York University in New York City, Ratnesh Lal of the University of California, Santa Barbara, and their colleagues found that β-amyloid, as well as several other proteins that produce similar deposits in various tissues, form channels in artificial membranes.

Yet not everyone is persuaded by the channel evidence. Glabe and his colleagues find that β-amyloid increases the permeability of both artificial and normal cell membranes, but this, he says, doesn't seem to depend on the formation of ion channels. In this case, β-amyloid's effects weren't specific; the protein increased the cross-membrane movements of both negatively and positively charged ions.

Glabe proposes that β-amyloid causes a generalized thinning of neuronal membranes. If that happens, he says, a cell would become leaky and have to work a lot harder to maintain normal internal ion concentrations. This could have a number of harmful effects, including the generation of reactive oxygen species, a normal but nonetheless cell-damaging byproduct of metabolism.

CREDIT: C. BICKEL/SCIENCE

The discrepancies between the two sets of observations remain unresolved. "I always assume we are both right. We're just not doing the same experiments," Glabe says. For the time being, other Alzheimer's researchers have taken something of a "wait-and-see" attitude about whether β-amyloid forms membrane channels for calcium ions. "No one has proved it with rigor that would allow it to become dogma, but no one has disproved it, either," says Gandy.

But there is another way in which β-amyloid may increase calcium entry into neurons: by altering the activity of the receptors that respond to stimulatory signals. Earlier this year, a team led by William Klein of Northwestern University in Evanston, Illinois, found that β-amyloid increases the calcium influx that occurs when the neurotransmitter glutamate activates the so-called NMDA receptor. Intriguingly, the researchers also found that memantine, a drug designed to inhibit NMDA receptor activity that has been approved for treating Alzheimer's, blocks this action of β-amyloid—an indication that drugs that restore calcium balance in neurons might indeed be therapeutic options for the disease.

From the inside

Whereas β-amyloid apparently affects calcium entry through the outer cell membrane, the presenilins exert their effects on an interior membrane. Calcium ions not only enter the cell from outside when a neuron is stimulated, but they are also released into the cytoplasm from internal stores, primarily from a membrane-bound compartment called the endoplasmic reticulum (ER). That's where the presenilins, which are located in the ER membrane, come in. "Presenilin mutations somehow cause a bigger calcium release from the ER when glutamate stimulates a cell," says Mark Mattson, whose team at the NIA Gerontology Research Center in Baltimore, Maryland, is one of several who made the finding.

This might be because calcium concentrations in the ER are elevated to begin with in cells bearing presenilin mutations. What causes that excessive accumulation has been unclear, but the answer may lie in new work from Ilya Bezprozvanny of the University of Texas Southwestern Medical Center in Dallas, Bart De Strooper of the Flanders Interuniversity Institute for Biotechnology (VIB4) and K. U. Leuven in Leuven, Belgium, and their colleagues.

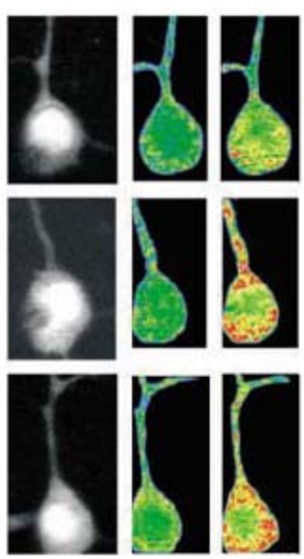
In experiments done over the past year or two, both on artificial membranes and on cultured nerve cells, they found that the normal presenilins are membrane channels that allow calcium ions to leak passively from the ER into the cytoplasm. However, presenilins carrying Alzheimer's mutations no longer function as calcium leak channels. Presenilin mutations "overload the ER with calcium, and you get excessive release on [nerve cell] stimulation," Bezprozvanny proposes. To Mattson, this sounds plausible. These results, he says, "seem to provide a molecular explanation for what we saw."

Early proponent. Zaven Khachaturian is a long-time advocate of the calcium hypothesis.

Other researchers, however, contend that presenilin mutations alter calcium handling in a different way. Frank LaFerla and his colleagues at UCI have looked at how presenilin mutations alter calcium release from the ER through two previously identified ion channels, known as the ryanodine and IP3 channels because they are activated by those chemicals. "When you stimulate either of them, you get a lot more calcium release in [PS] mutant cells than in normal cells," says LaFerla.

Through studies of mice genetically engineered with PS1 and other genes to develop Alzheimer's-like brain pathology, LaFerla, Grace Stutzmann, then a postdoc in his lab, and their colleagues found changes in the ER's handling of calcium occur in neurons even before the animals' brains developed the plaques and tangles characteristic of Alzheimer's. This finding, reported in the 10 May 2006 issue of the *Journal of Neuroscience*, indicates that the calcium changes might play a primary role in triggering neurodegeneration.

Some of the increased calcium release from the ER in PS-mutant cells may be due to greater expression of the ryanodine receptor, the LaFerla team has found. In as yet unpublished work, the researchers also observed that the presenilins are needed for the normal operation of the SERCA pumps that move calcium ions back into the ER after a neuron has fired. Not yet known is whether PS mutations affect SERCA pump operation. But if they increase it, the ER could become loaded with excess calcium ions.



All lit up. As indicated by the red color, neurons bearing mutant presenilins (*middle* and *bottom*) release much more calcium into the cytoplasm when stimulated than do normal neurons (*top*).

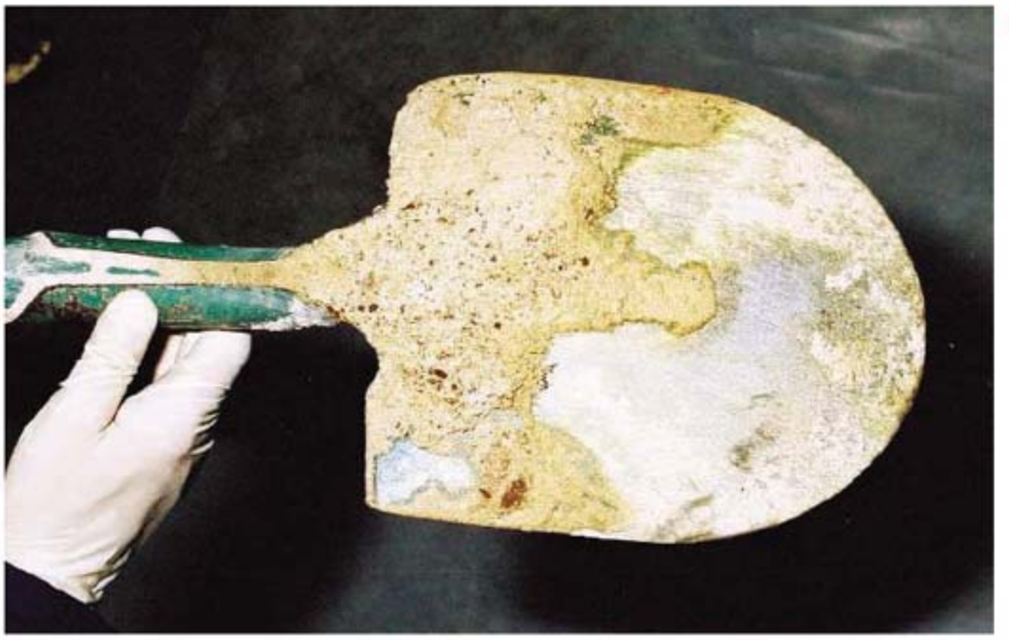
CREDITS (TOP TO BOTTOM): COURTESY OF ZAVEN KHACHATURIAN; STUTZMANN, ET AL., 2006, JOURNAL OF NEUROSCIENCE/SOCIETY OF NEUROSCIENCE

Mutations in the APP and presenilin genes together account for less than 10% of all Alzheimer's cases. The other 90%, mostly of the late-onset variety, fall into the so-called sporadic category, meaning that their causes aren't known. There are, however, indications that changes in calcium handling by neurons could be contributing to Alzheimer's susceptibility as we grow older. Some of this evidence comes from Olivier Thibault, Philip Landfield, and their colleagues at the University of Kentucky College of Medicine in Lexington.

In work reported early last year in the *Journal of Neuroscience*, these researchers looked at several indicators of calcium function in neurons obtained from the brains of rats at ages ranging from 4 to 23 months. Beginning at 12 months, which is middle age for rats, the neurons underwent several changes that should make them hyperexcitable, a response similar to that seen in cells with presenilin mutations. Changes such as these "could conceivably set the stage for Alzheimer's by making neurons more vulnerable to further insults," Landfield says. Those insults could include the increase in β-amyloid deposits that also occurs with age or membrane damage caused by reactive oxygen species.

Proving that similar calcium changes occur in humans could be difficult as researchers can't perform the same experiments on human brain neurons that Thibault and Landfield performed on rats. Consequently, the acid test of the calcium hypothesis in Alzheimer's disease will likely await possible clinical trials of drugs that inhibit calcium movements into the cytoplasm. That's "the only way to test cause and effect in sporadic Alzheimer's," Bezprozvanny says. Although researchers are beginning to test inhibitors of calcium release on cells in culture and animal models of Alzheimer's, it's still too early to tell whether they will find agents suitable for trials in humans.

–JEAN MARX



Case closed. Scientists traced soil on this shovel to the burial site of two murder victims.

FORENSIC SCIENCE

Dirty Science: Soil Forensics Digs Into New Techniques

Geologists, chemists, and other scientists are developing better ways of matching soil samples to help catch and convict criminals

A woman and her mother are reported missing from a township east of Adelaide in South Australia. The next day, the woman's car is found 160 kilometers away with a dirty, bloody shovel in the trunk. When her son shows up in a nearby town and tries to get assistance for the broken-down car, police arrest him. But the suspect refuses to talk, and with no bodies to provide evidence or even prove someone is dead, the desperate police seek help.

They call in a team of forensic soil scientists to analyze the shovel. The minerals, acidity, and moisture level of the soil on the shovel lead the team to suggest that the police search a gravel quarry in the Adelaide Hills, where days later a fox uncovers a body. The next day, the second body is found near the first. The son confesses to killing his mother and grandmother and is sentenced to 18 years in prison.

Although it could be a television episode of *CSI*, the case was real—and so were the soil scientists, who now work at the Centre for Australian Forensic Soil Science (CAFSS) in Adelaide, created in 2003 following the team's successful intervention in this 2000 double homicide. CAFSS analyzes soil for investigations from murder to environmental pollution, helps train new forensic scientists, and conducts research on new soil-analysis techniques. It has become well known among Australian detectives. "Ten years ago, police wouldn't have wanted to talk to us," says Rob Fitzpatrick, the center's director. "Now we can't cope with the number of cases."

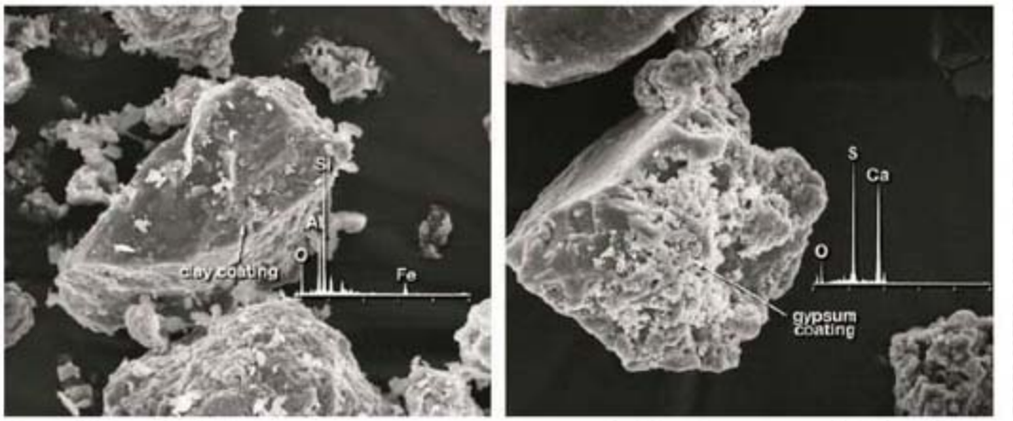
Soil evidence has been used to link criminals to crime scenes for more than a century. But in Australia and elsewhere, the recent automation of techniques and the ability to get information from smaller samples have made soil forensics an increasingly popular tool in criminal investigations. Scientists are now also exploring new ways of applying microscopy to dirt and of analyzing the plant waxes and microbial DNA within it.

Traditionally, soil forensics has been vulnerable to legal attack by defense lawyers because expert witnesses can testify only to whether samples are similar, versus the more absolute nature of a DNA or fingerprint match. Although some protocols are well-established—a soil sample is always sealed and locked, for example, and at least two people must be present while it's being analyzed—the field has yet to settle on the best means to analyze each soil type, explains Lorna Dawson of the Macaulay Institute in Aberdeen, U.K. One project aimed at standardizing old methods and validating new ones is the SoilFit project, led by Dawson and her colleagues. The effort also aims to provide a systematic database of soil fingerprints across the United Kingdom.

Reflecting the growing interest in applying new scientific techniques to soil, forensics researchers in Perth, Australia, last year hosted the first international conference on the topic, drawing several dozen attendees. This month, a second meeting in Edinburgh, U.K., is expected to bring together between 100 and 200 researchers, crime investigators, and forensic experts. "There's a lot of information in soil," says Dawson.

Fertile ground

Analyzing soil samples has a distinguished history in literature and real life. Sherlock Holmes uses soil to deduce Dr. Watson's peregrinations based on the dirt of his shoes in the 1890 work *The Sign of the Four*. A decade later, in the first known instance of soil evidence being used in a criminal investigation, German chemist Georg Popp helped authorities obtain a



Grounds for conviction? Scanning electron microscopy images of soil found on a suspect (*right*) and from a control (*left*) sample reveal differences on the microscale.

CREDITS (TOP TO BOTTOM): MICHAEL HEATH 2004 CSIRO; PHOTOS COURTESY OF EVELYNE DELBOS/MACAULAY INSTITUTE

confession in a murder case near Freiberg, Germany. Popp connected dirt from the trouser cuffs and fingernails of the main suspect to the crime scene.

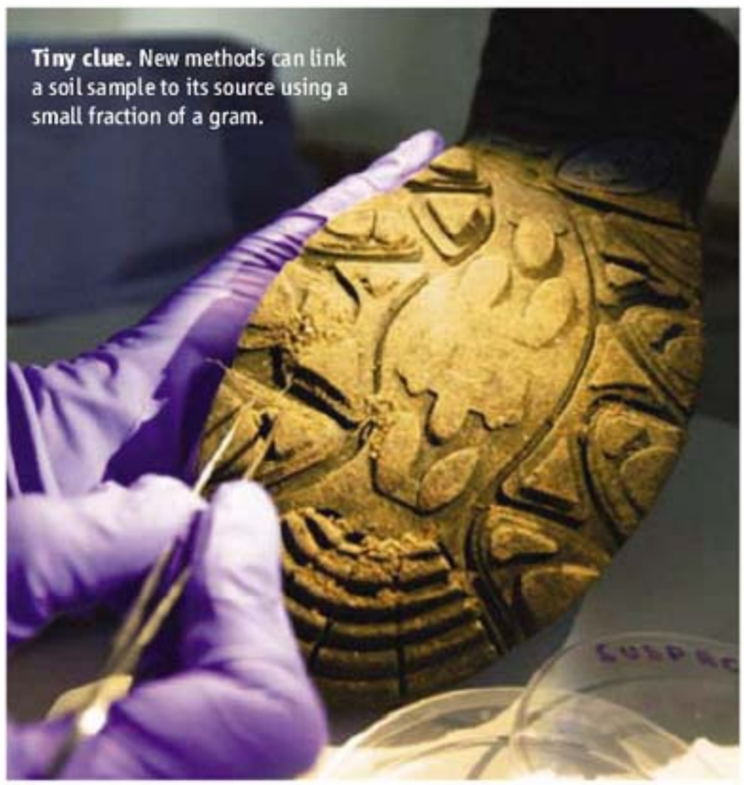
Matching soils is no small task. Soil is dynamic and part alive: A teaspoonful holds more than a million organisms, and soil microbes are constantly dying out or exploding in number. Water also leaches away compounds and introduces others as it trickles through. And soil is sensitive. Disturbing dirt—even by scooping a sample—changes it: Drying it alters its chemistry, exposing it to wind rounds out sharp edges on grains, and sealing it, such as in an evidence bag, can prompt a flurry of fungal growth. Such delicacy means that soil can only be pronounced in court as similar to or dissimilar from a possible source. Still, combining a few dirt characteristics can offer a compelling case for, say, linking a sample on a shoe to one in the back garden.

For the past few decades, soil scientists have used a variety of tools in criminal investigations. Ground-penetrating radar is able to pinpoint burial sites for individual bodies as well as mass graves. X-ray diffraction can uncover the minerals of the soil, infrared spectrometry determines the chemical pedigree, and analysis of diatoms and pollen provides biological clues to dirt's provenance.

Not all of those techniques can be applied to a given soil source, however. And others often require a greater sample size than the crime scene investigators can produce—hence the push for new, robust ways that require less dirt with which to work. As a visiting research fellow at CAFSS a few years ago, geologist Duncan Pirrie of the University of Exeter, U.K., saw how an automated scanning electron microscope could boost the availability and effectiveness of soil forensics. About 20 minerals occur in most soils, he explains, but what makes each sample identifiably distinct is the relative abundance of each mineral.

The CAFSS microscope, called QEMSCAN, finds both the mineral composition and its relative abundance from just 10 mg of dirt—50 times less than previously required. A similar instrument was originally developed for mining applications by Australian scientists, and the design was then adapted for use in forensic applications. QEMSCAN will analyze in 1 hour what would take a mortal days, and the scope's objective analysis triumphs over simple visual analysis of soils by people.

For a murder case in 2003, Pirrie hauled soil evidence from the United Kingdom to Australia for analysis, then promptly set up a QEMSCAN at his own university. Pirrie, who also conducts research on climate change in cretaceous Antarctica and on the effects of mining on coastal zones, says his lab is the only one in Europe with such a forensic scope. Today, the lab is called on about once a month to analyze traces of soil for murder and assault cases.



Tiny clue. New methods can link a soil sample to its source using a small fraction of a gram.

CREDIT: DAVID RILEY/MACAULAY INSTITUTE

Several new soil-analysis techniques remain a topic of lab research rather than court cases—at least for now. Organic substances among a soil's minerals can also offer an opportunity to match samples. One of Dawson's projects funded under the SoilFit umbrella looks at profiling soils by the mementos plants leave behind. Plants have a waxy covering to keep them waterproof. The mix of organic compounds—alkanes, acids, sterols, and other alcohols—is unique to each species and persists in the soil, sometimes for thousands of years. Dawson and colleagues are now refining a means of extracting the waxes to identify plants.

Jacqui Horswell, a soil microbiologist at the Institute of Environmental Science and Research in Porirua, New Zealand, is pursuing another means of matching soil samples: DNA. Millions of species of fungi and bacteria form complex communities in dirt, yet most remain unknown to scientists. Fewer than 1% of bacterial species can be cultured in the lab, she explains. But by applying a technique that chops DNA at specific target sequences and analyzes the length of the segments, Horswell can profile most of the bacteria in 200 mg of soil. The method doesn't identify individual species. Instead, without the need to culture any microbes, it produces a DNA signature for the organisms within the soil. Horswell and her research team published their first DNA soil profiles in 2001, and they hope that in another 5 years their database of soil DNA signatures will be large enough to be useful in court.

From science to law

Indeed, getting a new forensic technique established well enough for courts to recognize it can be a challenge. The SoilFit project, started in 2005 with funding from the U.K.'s Engineering and Physical Sciences Research Council (EPSRC), is one effort to give soil-matching more reliability as evidence. For prosecutors to better survive legal challenges in court, "we need a comprehensive survey of soil types in the United Kingdom" to substantiate the conclusions of an expert witness, says Derek Auchie, director of undergraduate law programs at Aberdeen Business School. To that end, EPSRC gave Dawson's team a £350,000 grant to analyze all feasible combinations of soil types—such as loams, peat, and alluvial soils—and vegetation such as grassland, heather, and forest. To date, they have tested an array of analysis techniques on all 120 combinations and are now comparing each technique's accuracy to work out which ones work better for which soil combinations.

EPSRC funded SoilFit under its Crime Initiative, which seeks to bridge crime-fighting services and academic research to benefit U.K. citizens. The project is "developing a community of researchers active in [fighting] crime," says Peter Hedges, head of EPSRC's Economy, Environment and Crime Team. Dawson predicts that the SoilFit database will be ready for detectives and prosecutors in 2008. Sherlock Holmes would be pleased. **–KRISTA ZALA**

Krista Zala is a freelance writer in Los Angeles, California.

Maritime feat. The first boats may have been made of bamboo.

ARCHAEOLOGY

In Search of the World's Most Ancient Mariners

Researchers debate the capabilities of the first human voyagers, who traveled the waters of Southeast Asia at least 45,000 years ago

CAMBRIDGE, U.K.—We humans are terrestrial animals, yet we spend a lot of time gazing wistfully over bodies of water. We flock to the seashore or the lakeside at the slightest sign of mild weather and celebrate the romance of the sea in art and literature. Early seafaring was central to the spread of civilization, and today thousands of vessels ply the world's oceans, searching for fish and hauling billions of tons of cargo.

Despite the importance of seafaring to culture, however, archaeologists are not sure how, when, and why humans first ventured into the oceans. The earliest known boats, hollowed out logs found in the Netherlands and in France, are at most 10,000 years old. And the earliest indirect evidence for sea crossings in Europe—human occupation of Cyprus and the Greek island of Milos—dates to only 12,000 to 13,000 years ago. Yet ancient archaeological sites in present-day Australia, Indonesia, and other Southeast Asian islands suggest sea crossings at least 45,000 years ago, soon after modern humans first left Africa.

At a meeting here last month,* three dozen archaeologists and maritime historians sifted through the evidence for seafaring through the ages. They debated, sometimes sharply, whether the earliest mariners crossed the sea purposely or by accident. "There is a danger in accepting either of these extreme positions," says William Keegan, an anthropologist at the Florida Museum of Natural History in Gainesville. "But I have no problem believing that people who were exploiting coastal resources had developed the ability to cross the water gaps in question by 50,000 years ago."

The meeting also heard dire warnings that rising sea levels—which are already at least 50 meters higher than when modern humans first took to the oceans—might put evidence crucial to resolving these questions out of reach. "There are drowned terrestrial landscapes that were occupied by our ancestors," says archaeologist Jon Erlandson of the University of Oregon in Eugene. "But we know almost nothing about them."

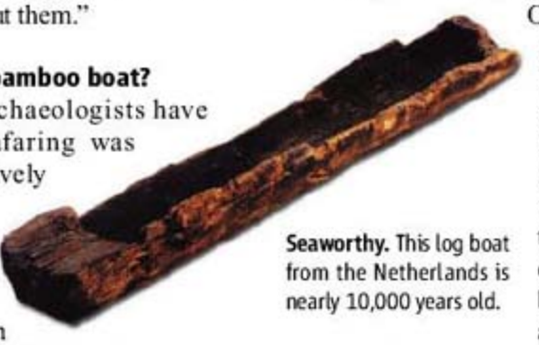
Blown about in a bamboo boat?

Although most archaeologists have assumed that seafaring was invented by cognitively advanced modern humans, one earlier hominid seems to have jumped the gun. In 1998, a team led by archaeologist Michael Morwood of the University of New England in Armidale, Australia, dated stone tools on the Indonesian island of Flores to 800,000 years ago, when *Homo erectus* was known to inhabit the Southeast Asian mainland. The occupation of Flores almost certainly required a sea crossing, and Morwood suggested at the time that the cognitive abilities of *H. erectus* might be "due for reappraisal" (*Science*, 13 March 1998, p. 1635.)

Yet the lack of other evidence anywhere near so early suggests to many researchers that this was a fluke that did not require technology. Perhaps a small band of hominids was blown out to sea on floating vegetation, as occasionally happens to other mammals who then found island populations. The possibility that *H. erectus* evolved in isolation on Flores for thousands of years, eventually becoming the tiny *H. floresiensis*, a.k.a. the Hobbit, supports the rarity of traveling to or from Flores.

"Flores is the exception that proves the rule in terms of when seafaring really began," says Atholl Anderson, a prehistorian at the Australian National University (ANU) in Canberra. Erlandson agrees: "Otherwise, *H. erectus* should have colonized Australia and the surrounding islands." Yet although the trek to Australia could be accomplished by relatively short hops across a multitude of islands, there is no evidence that *H. erectus* ever made that journey. Modern humans were the first hominids in Australia, arriving no earlier than 60,000 years ago, and many archaeologists are skeptical of dates earlier than 45,000 years. Even then, it's hard to differentiate true seafaring from a bit of boating gone wrong, says archaeologist Geoff Bailey of the University of York in the U.K. "It remains an open question whether the move into Australia was a purposeful, high-tech exercise in skilled navigation or a low-tech process of almost accidental drift that resulted in the opening up of a maritime universe."

Both viewpoints were in evidence at the meeting. In her talk, ANU archaeologist Susan O'Connor argued that modern humans did not necessarily require sophisticated seafaring skills to colonize Australia and nearby islands. She proposed that early humans traveled by simple bamboo rafts—probably already used to explore rivers and estuaries—then drifted out to sea and were blown about by the monsoon.



Seaworthy. This log boat from the Netherlands is nearly 10,000 years old.

And island hopping was easier in the past. About 45,000 years ago, sea levels were roughly 50 meters lower than they are today. As a result, Australia, New Guinea, and

*Global Origins and Development of Seafaring, Cambridge, U.K., 9–12 September 2007.

CREDITS (TOP TO BOTTOM): CARL AND ANN PURCELL/CORBIS; DRENTS MUSEUM COLLECTION

Tasmania formed a single continent known as Sahul, whereas Borneo, Java, and the Malay Peninsula were joined together in a continental shelf called Sunda (see map). Although the earliest dates for modern human occupation of Sahul are controversial, excavations on several islands north of Sahul have produced radiocarbon dates of up to 45,000 years ago—including O'Connor's own excavations at Jerimalai Cave on East Timor, which recently clocked in at 42,000 years. If Sahul was colonized as early as 60,000 years ago, O'Connor contended, then humans' fairly leisurely spread supports a more accidental than purposeful journey.

O'Connor concluded that when the colonizers did venture farther out to sea, traveling 180 kilometers to the islands of Buka by 28,000 years ago and 230 kilometers to Manus by 21,000 years ago, their earlier seafaring experience might have "preadapted" them to later innovations in boating technology, including larger vessels made of wood and the use of sails. Nevertheless, O'Connor and others stressed, there is no direct archaeological evidence for the use of sails that early, indeed none at all before about 7000 years ago in the Near East.

Changing seascape. Lower sea levels exposed more land during glacial periods (shown here at 22,000 years ago) and made ocean crossings easier.

The short chronology

O'Connor's scenario, which archaeologists call the "long chronology" for the colonization of island Southeast Asia, was challenged at the meeting by archaeologist James O'Connell of the University of Utah in Salt Lake City. In the last few years, O'Connell, together with archaeologist Jim Allen of La Trobe University in Bundoora, Australia, has argued from a detailed analysis of radiocarbon dates for a "short chronology" that puts the occupation of Sahul no earlier than about 50,000 years ago. He pointed out that by 45,000 years ago modern humans had colonized a number of islands between Sunda and Sahul, called the Wallacean Archipelago, which stretched at least 1000 kilometers even when sea levels were at their lowest. Reaching many of these islands required sea crossings of 30 to 70 kilometers, sometimes against the currents. Most animals from Asia never achieved these crossings, implying that humans must have used technology to do it. That 5000 years of colonization, O'Connell said, represented a relatively short "archaeological instant." Rather than drifting, O'Connell argued, early seafarers must have had "marine-capable watercraft" and keen navigation skills.

To bolster his argument, O'Connell pointed out that remains of open-ocean fish, including tuna and sharks, have been found at numerous island sites dating more than 40,000 years ago, an indication that the colonizers already had boats capable of deep-sea fishing.

O'Connell also cited recent demographic simulations by anthropologist John Moore of the University of Florida in Gainesville and others, suggesting that successful colonizations require a minimum founder group of 5 to 10 women of reproductive age and a similar number of men. "The odds that the members of a small group cast adrift by chance, then tossed up on an isolated shore, could generate a successful population are long indeed," O'Connell concluded.

The conflicting talks drew varied reactions. "My tendency would be to side with [O'Connell]," says Keegan. "For me the issue is what was socially possible. Humans live in groups, and successful colonists tend to reproduce those groups. They have a better chance of survival if they can maintain contact with their parent community," for example, by making return sea voyages back home. But Anderson counters that the relatively mild, tropical conditions around Sahul 45,000 years ago and the abundance of species of giant, wide-diameter bamboo, perfect for making rafts, ensured that accidental voyagers would survive at sea. "If people were habitually using bamboo [rafts] to explore coral reefs and lagoons, and if they did so as family groups, then the chance of an accidental passage was always there." Moreover, Anderson says, even such simple craft were capable of carrying a "viable colonizing group of 5 to 10 people" and could be blown across the sea "within a few days."

Bailey notes that "island Southeast Asia offers all the right conditions for just such a gradual process," including warm seas and "lots of very productive marine resources like fish, sea mammals, turtles, and shellfish, which would have encouraged exploration of offshore islands."

Indeed, Bailey suggests that the special conditions in Southeast Asia might explain why the earliest evidence of seafaring is there rather than in the Mediterranean, where seafaring only shows up about 13,000 years ago—even though modern humans occupied southern Europe beginning at least 40,000 years ago. "The Mediterranean offers a stark contrast," Bailey says. "When it comes to marine fertility and productivity of offshore resources, it is very nearly at the bottom of the world league, with little tidal movement … and temperature gradients that trap nutrients on the seabed below the zone of photosynthesis." Erlandson agrees: "One of the take-home messages of the meeting was that the development of seafaring capabilities was not universal, but was contingent on a variety of ecological and cultural conditions."

The other take-home message, Erlandson says, is that the current rise in sea levels caused by global warming, and the accelerated erosion of coastlines, "is threatening our best source of information about such conditions." Because ancient boats would have been launched from shores now underwater, the best chance of finding evidence for them lies in exploring coastal sites where the ancient shoreline is near the present one, for example, where the land falls off steeply into the sea. Yet most of these sites, Erlandson says, "are actively eroding and countless others have already been destroyed. Enormous amounts of information will be lost in coming decades unless we find, date, and excavate them." **–MICHAEL BALTER**

SOURCE: ADAPTED FROM PETER WHITE AND JAMES O'CONNELL

Public health response 396

Limitations of genetic ancestry testing 399

LETTERS

edited by Jennifer Sills

Of Aging Mice and Men

LIU *ET AL.* (REPORT, "AUGMENTED WNT SIGNALING in a mammalian model of accelerated aging," 10 August, p. 803) have elegantly shown how alterations in Wnt signals contribute to the suffering of klotho-deficient mice, but not every sick little rodent is a suitable model for human aging. The pathological features and short life span of klotho mutant mice have been shown to reflect hypervitaminosis D, secondary to ablated responses to Fgf-23 (*1*–*3*). The same syndrome appears in Fgf-23 mutants and can be cured by deleting the 1-α-hydroxylase gene that increases the activity of the vitamin. In both mutants, the features represented as evidence of "premature aging" can be eliminated simply by putting the mice on a diet low in vitamin D. Perhaps vitamin D deprivation will turn out to be the long-sought cure for aging, but in the meantime, it would be wise to view with some skepticism the claims that klotho and similar developmental mishaps provide convenient shortcuts for learning about mechanisms of "real" aging.

RICHARD MILLER

Geriatrics Center and Department of Pathology, University of Michigan, Ann Arbor, MI 48109–2200, USA.

References

1. M. S. Razzaque, B. Lanske, *Trends Mol. Med.* **12**, 298 (2006).
2. H. Tsujikawa, Y. Kurotaki, T. Fujimori, K. Fukuda, Y. Nabeshima, *Mol. Endocrinol.* **17**, 2393 (2003).
3. B. Lanske, M. S. Razzaque, *Ageing Res. Rev.* **6**, 73 (2007).

Response

THERE ARE MANY AREAS IN AGING RESEARCH IN which there is some disagreement. One question in dispute is the degree to which observations in simple organisms, such as postmitotic worms, can inform our understanding of mammalian aging. Similarly, reasonable people disagree on the role, if any, of cellular senescence in organismal aging. We appreciate that there is also considerable disagreement regarding how much mammalian models of accelerated aging can teach us about the normal aging process.

Our study centered on a set of observations suggesting that the Wnt family of proteins could bind to klotho, a protein whose absence has been linked to an accelerated aging phenotype in mice. Genetic evidence suggests that alleles of klotho are also associated with variation in human longevity (*1*). Nonetheless, we agree with Miller that considerable care must be taken when using the existing accelerated aging models as an indication of the normal aging process. Our opinion is that studying models of rapid aging will be useful in teasing out the underlying mechanisms of how we age, although we understand that Miller does not share that opinion. Hopefully, we will all live long enough to find out who is right.

HONGJUN LIU AND TOREN FINKEL

Cardiology Branch, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20892, USA.

Reference

1. D. E. Arking *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 856 (2002).

Replicating Genome-Wide Association Studies

GENOME-WIDE ASSOCIATION STUDIES PROMISE to significantly expand our knowledge of host control of deadly pathogens. Lurking in the background of these studies, however, is a serious methodologic issue. Individuals who participate in the cohorts used in genome-wide association studies are often ethnically and racially different from their fellow citizens who do not participate in these studies (*1*); more important, they are markedly different from the populations of developing countries with the highest burdens of infectious diseases.

The Report "A whole-genome association study of major determinants for host control of HIV-1" (J. Fellay *et al.*, 17 August, p. 944) demonstrates how much can be learned from the study of a highly motivated, largely European cohort. Unfortunately, rather than suggesting that readers strive to replicate the study findings in different populations, J. Fellay *et al.* instead proceed directly to discussion of "directions for therapeutic intervention" and "urgency in carrying out similar studies for other infectious diseases."

In rushing these issues, the authors overlook several important points. Similar studies conducted in different geographic regions may fail to find the same associations, and may even find different associations. The highly polymorphic nature of human MHC, different pathogen strains, or gene-environment interactions could all result in variability of associations across populations in different regions. In some cases, such as the CCR5-Δ32 mutant allele, genetic associations specific to geographic region may indeed aid drug or vaccine target discovery (*2*). However, a premature focus of financial and intellectual resources on a few specific alleles may throw out the baby for the bathwater.

MARK H. KUNIHOLM

Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA.

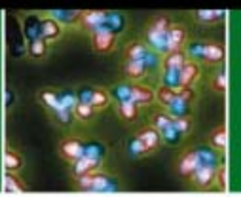
References

1. A. L. Gifford *et al.*, *N. Engl. J. Med.* **346**, 1373 (2002).
2. J. A. Este, A. Telenti, *Lancet* **370**, 81 (2007).

CREDIT: JOE SUTLIFF

Scooping up the solar wind

401



Why quirky genomes?

405

Response

WE RECENTLY REPORTED THAT THREE POLYmorphisms significantly influence host response to HIV-1. Two of these polymorphisms associate with viral load during the asymptomatic set point period, and the third associates with a measure of HIV-1 disease progression.

Kuniholm raises the question of whether the associations could be "replicated" in other geographic regions and suggests that it would have been preferable to evaluate this rather than moving to practical applications of the findings.

Our original study included samples from multiple European populations, both north and south; the effects observed cannot be viewed as the result of a specific cohort or geographic region within Europe. In the original study, we also replicated all three discoveries in a fully independent set of samples.

Kuniholm is correct that genetic effects are sometimes observed in some population groups and not others. The current consensus view is that when a polymorphism is present in different geographic regions, it tends to have a similar effect, but causal variants do vary in frequency among different groups (*1*, *2*). Indeed, one of our associations is known to be rare or absent in some geographic regions. Absence of a relevant genetic variant in a particular population does not in itself limit the applicability of new knowledge: The example of the CCR5-Δ32 variant illustrates this point by demonstrating that a medication of universal use can indeed be developed on the basis of genetic information from one human population. The question of the geographic distribution of causal polymorphisms is an important one, but it is separate from the question of whether the polymorphisms have important clinical effects in the groups under study. Indeed, we are currently expanding our study to include multiple cohorts from the United States and from Africa.

DAVID B. GOLDSTEIN

Center for Population Genomics and Pharmacogenetics, Duke Institute for Genome Sciences and Policy, Duke University, Durham, NC 27710, USA.

References

1. J. P. Ioannidis, E. E. Ntzani, T. A. Trikalinos, *Nat. Genet.* **36**, 1312 (2004).
2. D. B. Goldstein, J. N. Hirschhorn, *Nat. Genet.* **36**, 1243 (2004).

Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

A Measure of Respect for Translational Research

IT WAS A PLEASANT SURPRISE TO SEE A SPECIAL feature ("Careers in translational research," 17 August, p. 966) in *Science* focusing on translational research and its opportunities, risks, and challenges. In their respective articles, S. Carpenter (p. 966) and K. Garber (p. 968) highlight the concerns that translational researchers have about not being able to satisfy traditional measures of scientific success, including number of publications and impact factors. This apprehension is well founded. More worrisome is the scenario in which the onus is placed on the members of the translational community to prove their worth. I think it is too much. Measures of basic research productivity are well established, but the same is not true for translational research. I agree with Wu's advice (p. 967) to "go with what you passionately care about, because it's a long row, no matter how you hoe it," but I also sympathize with June's lament (p. 969): "I have seen several instances since I've been at [Penn] where promising translational researchers had to go back and just do basic research in order to assure their promotion."

It is time to think seriously about how to develop criteria for quantitatively evaluating

translational work. "Bench-to-bedside" and "lab-to-clinic" research will otherwise suffer from a perennial problem of lack of recognition. Considering the risk of failure in translational research, we need to be open-minded and adopt measures that focus not only on success but on honest effort. Achievements such as partnering, patents, clinical trials, and drug screening should be considered on par with publications for assessment and promotion. Successes in translational efforts should be provided with "impact factors" commensurate with the volume of work, time taken, or importance in terms of clinical or pharmacological utility.

ABHAY SHARMA

Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India.

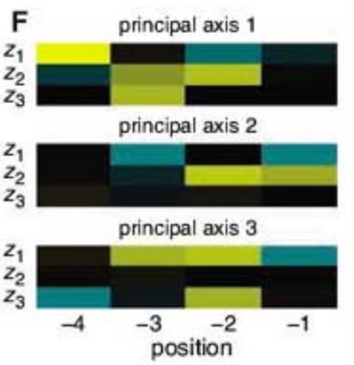
CORRECTIONS AND CLARIFICATIONS

News Focus: "Accidents spur a closer look at risks at biodefense labs" by J. Kaiser (28 September, p. 1852). The highest biocontainment level is "biosafety level 4," not "biosecurity level 4," as stated in the article.

News of the Week: "Lapses in biosafety spark concern" by J. Couzin (14 September, p. 1487). A report by the Centers for Disease Control and Prevention (CDC) incorrectly noted that its last inspection of Texas A&M University's biosafety program prior to July had been in February 2007. CDC has since noted that this was a typo. The inspection took place in February 2006.

Special Issue on Attosecond Spectroscopy: Reviews: "The future of attosecond spectroscopy" by P. H. Bucksbaum (10 August, p. 766). In the second line of the legend to Fig. 1, the phrase "two cojoined coins" should read "two cojoined cones."

Reports: "PDZ domain binding selectivity is optimized across the mouse proteome" by M. A. Stiffler *et al.* (20 July, p. 364). The position numbers appeared in the wrong order in Fig. 3F. The corrected panel is shown here.



Reports: "Genome plasticity a key factor in the success of polyploid wheat under domestication" by J. Dubcovsky and J. Dvorak (29 June, p. 1862). In the final reference, National Research Institute should have been National Research Initiative.

Reports: "Thrice out of Africa: Ancient and recent expansions of the honey bee, *Apis mellifera*" by C. W. Whitfield *et al.* (27 October 2006, p. 642). Several critical references were left out of the final manuscript. In discussing the fact that "ample evidence shows that both European and African alleles occur in Africanized populations" (p. 644), we should have referenced two studies that first demonstrated introgression between invading Africanized and resident European honey bees in Texas: M. Pinto *et al.*, *Evolution* **58**, 1047 (2004) and M. Pinto *et al.*, *Genetics* **170**, 1653 (2005). In addition, these studies showed no differences between mitotypes of Africanized and European bees in the later years of Africanization. References to this conclusion should have been cited at the end of the first paragraph on p. 645. We greatly regret that these references were omitted, and for this we extend our apologies to Pinto *et al.* The North American portion of this effort was built upon the Pinto *et al.* work. It was only because we could make use of many of the same bees used in the Pinto *et al.* study that we were able to corroborate the results of Pinto *et al.* and then expand on them, showing that the lack of correlation between mtDNA and nuclear DNA involved markers distributed throughout the nuclear genome, and examining in more detail the relationships between M-, C-, O-, and A-derived genomes.

TECHNICAL COMMENT ABSTRACTS

Comment on "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension"

Nader Sanai, Mitchel S. Berger, Jose Manuel Garcia-Verdugo, Arturo Alvarez-Buylla

Curtis *et al.* (Research Articles, 2 March 2007, p.1243) claimed discovery of a human neuronal migratory stream to the olfactory bulb along a putative lateral ventricular extension. However, high levels of proliferation reported with proliferating cell nuclear antigen were not confirmed using different markers, neuronal chain migration was not demonstrated, and no serial reconstruction shows a true ventricular extension.

Full text at www.sciencemag.org/cgi/content/full/318/5849/393b

Response to Comment on "Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension"

Maurice A. Curtis, Monica Kam, Ulf Nannmark, Richard L. M. Faull, Peter S. Eriksson

In contrast to a previous study of Sanai *et al.*, our study had the advantage of using serial sagittal sections of the human basal forebrain, combined with 5-bromo-2′-deoxyuridine labeling, rigorous magnetic resonance imaging, and polymerase chain reaction analysis. We believe these methods convincingly demonstrate the presence of a rostral migratory stream in the human brain that resembles that in other mammals.

Full text at www.sciencemag.org/cgi/content/full/318/5849/393c

ECONOMICS

Genetically Capitalist?

Samuel Bowles

A Farewell to Alms
A Brief Economic History of the World
by Gregory Clark
Princeton University Press, Princeton, NJ, 2007. 432 pp. $29.95, £17.95. ISBN 9780691121352.

Joseph Townsend's 1786 broadside against England's poor laws tells the story of a South Seas island on which the Spaniards had placed a few goats that eventually overran the island, their numbers and starvation fluctuating in tandem. English pirates preyed both on the goats and on Spanish shipping, so eventually the Spaniards introduced a pair of greyhounds, hoping to eliminate the goats. As greyhounds multiplied and the goat population crashed, hunger overtook the greyhounds. The goat population revived, and "a new kind of balance was established." Townsend's point: "The course of nature may be easily disturbed, but man will never be able to reverse its laws." As a result, governments' attempts to elevate the poor were "absurd" and "impractical" (*1*).

Townsend anticipated Thomas Malthus's *Essay on the Principle of Population* (*2*) by more than a decade. Gregory Clark's *A Farewell to Alms* continues this tradition. On the cover, a ghoulish begging hand reaches toward the reader.

Clark is an economic historian (at the University of California, Davis) whose quantitative studies are highly regarded. He calls his book "an unabashed attempt at *big history*, in the tradition of *The Wealth of Nations*, *Das Kapital*, *The Rise of the Western World*, and … *Guns, Germs, and Steel*." Clark seeks to explain why sometime "around 1800" England but not other parts of the world broke out of the Malthusian trap illustrated by Townsend's goats and greyhounds, and why economic stagnation persisted even into the 21st century in some parts of the world. "Then," he adds, "we will understand the history of mankind."

The puzzle of England's take-off has challenged generations of scholars (*3*–*5*). If a consensus exists today, it echoes both Adam Smith and Karl Marx: institutions made the difference, whether limited government, competition for profits, the expansion of markets, secure property rights, the enclosure of common lands, or empire. Clark dissents from this view and provides a number of telling counterarguments. Building on the ideas of Oded Galor and Omer Moav (*6*), he proposes that it was not institutions but people that changed and that their new values—"thrift, prudence, negotiation, and hard work"—led them to save, work, and invest in ways that would eventually bring about the industrial revolution.

This theme is reminiscent of Max Weber, who, in *The Protestant Ethic and the Spirit of Capitalism* (*7*), held that by transforming profit seeking from a moral weakness to a personal duty, Calvinism became capitalism's midwife. The idea that differences in values might explain societal differences or historical change never penetrated economics. A widely accepted, if empirically implausible (*8*), methodological fiat due to Gary Becker and George Stigler held that "one does not argue about tastes for the same reason that one does not argue about the Rocky Mountains—both are there, and will be there next year, too, and are the same to all men" (*9*). Recent advances in experimental economics have challenged the fiat (*10*), but Clark is nonetheless swimming against the current.

Unlike Weber, for Clark the lever that changed values was not religious conversion but biology: the rich enjoyed higher fitness than the rest and their "capitalistic attitudes" spread as a result. Clark's companion paper "Genetically capitalist?" (*11*) sums it up: "The triumph of capitalism in the modern world thus may lie as much in our genes as in ideology or rationality."

Here is the argument: (i) "unusually in England," from 1250 on rich commoners had more surviving children than the rest; (ii) the children of the rich also became rich and had higher-than-average reproductive success; (iii) the distinctive values that accounted for their economic success would eventually propel the industrial revolution; (iv) these values were transmitted to their descendants either culturally or "perhaps" genetically; (v) and therefore proliferated; (vi) eventually springing England from the Malthusian trap.

Clark's own research documenting the reproductive success of wealthy Englishmen (i) and the tendency of their offspring also to be rich (ii) is convincing. But was this really unusual? Rich commoners outproduced the poor throughout early modern Europe and in other pre-industrial societies (*12*). Clark's only evidence that this was not the case in Japan and China concerns samurai and Qing nobility. But English nobles, too, had lower-than-average reproductive success prior to the 18th century (excessive dueling). So the Japanese and Chinese data do not support Clark's claim. The link between parental and offspring wealth was not uniquely English (*12*).

Georges de la Tour's *St. Joseph, the Carpenter* (1640s).

CREDIT: BRIDGEMAN ART LIBRARY

Personality differences contribute to individual differences in economic fortunes, but hard evidence for the particular set of values implied by (iii) is intrinsically hard to come by and Clark provides none. Data from modern economies suggest that personality influences individual success, but the effects are quite modest (*12*–*14*).

Parents transmit personality traits to their children, and there is good evidence that genetic transmission is involved for some social behaviors (*12*, *15*, *16*). However, none of this evidence con-

The reviewer, the author of *Microeconomics: Behavior, Institutions, and Evolution*, is at the Santa Fe Institute, 1399 Hyde Park Road, Santa Fe, NM 87501, USA, and in the Department of Political Economics, University of Siena. E-mail: bowles@santafe.edu

cerns hard work, patience, or the other values that Clark stresses. And the correlations between parental and offspring measures of personality are strikingly low. John Loehlin's survey of 859 such correlations found a mean value of 0.13—and the correlation for the personality dimension most relevant to Clark's argument ("conscientiousness") is even lower: 0.09 (*17*). Thus whether genetic or cultural, parental influence on descendent preferences is quickly dissipated across the generations, which makes point (iv) unlikely.

Clark's evidence that interest rates and interpersonal violence declined and that Londoners in 1800 worked long hours (by comparison with hunter-gatherers) did not convince me that (v) is true. A more serious shortcoming concerns (vi). The behavioral foundations of the incessant and cumulative innovation that made the industrial revolution are more plausibly to be found in Joseph Schumpeter's Dionysian entrepreneurial types than in a workaday penchant for diligence, prudence, and patience.

But let's ignore the fact that the world is full of prudent, hardworking, and patient people who nonetheless remain poor and suppose that these dispositions explain both individual and societal economic success. If from 1250 or even earlier these "capitalistic" values were spreading as the surplus children of the rich cascaded down the social ladder, why do we not observe a gradual acceleration of the economy beginning in the 13th century rather than the abrupt take-off that Clark documents occurring more than half a millennium later? And why did the equally capitalistic Netherlands not also take off? The argument thus explains neither the location nor the timing of the first escape from the Malthusian trap.

Clark's barbs at economists and the World Bank reflect his view that their prescription for poverty—"getting the institutions right"—is less important than people getting their values right. Clark also favors less-restrictive immigration policies. Along with the suggested genetic explanation, Clark's pull-up-your-socks message to today's poor (as it will inevitably be read) ensures both controversy and a wide readership.

A Farewell to Alms asks the right questions, and it is full of fascinating details, like the speed at which information traveled over two millennia (prior to the 19th century, about one mile per hour). Clark's combination of passion and erudition makes his account engaging. When a light bulb goes off in my head, the first thing I ask myself is "Would this be interesting if it were true?" Clark's thesis definitely meets that test.

But I doubt that it is true. Clark anticipated this reaction in his preface: "far better such ["controversial"] error than the usual dreary academic sins."

References and Notes

1. J. Townsend, *A Dissertation on the Poor Laws by a Well Wisher to Mankind* (Univ. of California Press, Berkeley, CA, 1971); http://socserv.mcmaster.ca/econ/ugcm/3ll3/townsend/poorlaw.html.
2. T. R. Malthus, *An Essay on the Principle of Population, as It Affects the Future Improvement of Society: With Remarks on the Speculations of Mr. Godwin, M. Condorcet, and Other Writers* (J. Johnson, London, 1798).
3. R. Allen, *Enclosure and the Yeoman* (Clarendon, Oxford, 1992).
4. R. Brenner, *Past Present* **70**, 30 (1976).
5. K. Pomeranz, *The Great Divergence: China, Europe, and the Making of the Modern World Economy* (Princeton Univ. Press, Princeton, NJ, 2000); reviewed by G. Lang, *Science* **288**, 982 (2000).
6. O. Galor, O. Moav, *Q. J. Econ.* **117**, 1133 (2002).
7. M. Weber, *The Protestant Ethic and the Spirit of Capitalism*, T. Parsons, Transl. (Allen and Unwin, London, 1930).

8. S. Bowles, *J. Econ. Lit.* **36**, 75 (1998).
9. G. S. Becker, G. J. Stigler, *Am. Econ. Rev.* **67** (2), 76 (1977).
10. J. Henrich *et al.*, *Behav. Brain Sci.* **28**, 795 (2005).
11. G. Clark, "Genetically capitalist? The Malthusian era, institutions and the formation of modern preferences"; www.econ.ucdavis.edu/faculty/gclark/papers/Capitalism%20Genes.pdf.
12. See S. Bowles, www.santafe.edu/~bowles/clark.pdf.
13. S. Bowles, H. Gintis, M. Osborne, *J. Econ. Lit.* **39**, 1137 (2001).
14. J. Heckman, J. Stixrud, S. Urzua, *J. Labor Econ.* **24**, 411 (2006).
15. S. Bowles, H. Gintis, *J. Econ. Perspect.* **16** (3), 3 (2002).
16. B. Wallace, D. Cesarini, P. Lichtenstein, M. Johannesson, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 15631 (2007).
17. J. Loehlin, in *Unequal Chances: Family Background and Economic Success*, S. Bowles, H. Gintis, M. O. Groves, Eds. (Princeton Univ. Press, Princeton, NJ, 2005), pp. 192–207.

10.1126/science.1149498

PUBLIC HEALTH

A Crisis Is a Terrible Thing to Waste

Paul S. Keim

With 9/11 setting the stage for the anthrax letter attacks, many of us in the United States were too busy to analyze the impact of this crisis on public health. We were, in fact, responding to the crisis in an all-too-consuming manner. As the adrenaline rush and crushing work load lightened, many U.S. biodefense leaders began to design a road map for infectious disease and public health efforts. The 2001 terrorist attacks provided the political impetus to create a sustainable biodefense infrastructure and skilled workforce for the long-term benefit of public health in the United States. Government is notorious for impulsive spending sprees that fade with a changing political environment. Was this for real, or the latest Washington knee-jerk reaction?

In *Are We Ready?* David Rosner and Gerald Markowitz revisit the events and actions of that time to determine if we have, indeed, wasted the opportunity to do something sustainable and with a long-term impact on public health. Rosner (a professor of public health and history at Columbia University) and Markowitz (a professor of history at the City University of New York) do this through extensive interviews of the individuals involved in New York City, as well as officials at the state and national levels, followed by analysis and recommendations. Their approach is based on largely anecdotal evidence, but they offer an impressive amount that is supported by numerous citations and interviews. The interviews are interwoven with a historical perspective and analysis, making for a compelling review.

Are We Ready?
Public Health Since 9/11

by David Rosner and Gerald Markowitz

University of California Press, Berkeley, and Milbank Memorial Fund, New York, 2006.
210 pp. $45, £26.95.
ISBN 9780520249202.
Paper, $16.95, £9.95.
ISBN 9780520250383.
California/Milbank Books on Health and the Public

The book covers the chaos in New York City following 9/11 and the anthrax letters incidents and how these events shifted priorities of public health. Not surprisingly, the authors document that the effects encompassed every aspect of life in the city. From high school administrators to the governor, uncertainty about the dangers and responsibilities was common. But so too were tales of leadership, coordination, and unselfishness—such as the story of seniors, who had lived through previous disasters and wars, comforting their caregivers. While the political leadership played a role, Rosner and Markowitz are more skeptical than past and current sound bites about its importance. Their presentation places the events in the context of New York political and social history. They conclude that the effectiveness of New York's response was only partially due to the contemporary political leadership and more due to institutional structures built over many years.

Long-neglected state public health departments were suddenly in the limelight after 9/11, with newfound importance to their governments and citizens. Ronald Cates of the Missouri Department of Health and Senior Services noted that "A lot of people who couldn't spell 'public health' now saw public health as the equivalent of the Department of Defense." Yet, as the excitement faded, the reality remained that federal funding was often targeted for highly specific bioterrorism projects (e.g., smallpox vaccination), while routine essential services were floundering due to a lack of resources. Local experiences across the country were uneven, with some states managing the influx of resources well while others did not. The 9/11-induced (or at least -accelerated) economic recession decreased state revenues—decreases that were invariably passed on to the state agencies. In some cases, this furthered the disparity between federally funded bioterrorism programs and traditional public health services. Many of the numerous experiences recounted by the authors document the states' struggles to "dually use" the bioterrorism funding both for biodefense and to strengthen the overall public health infrastructure.

The failed smallpox vaccination program initiated in December 2002 was driven by federal priorities yet had to be implemented at the state and local levels. To state officials, the true nature of the threat was not obvious. As a result, many of them did not fully engage in a program that was funded at less than its true implementation cost. In addition, the public did not fully recognize the threat, and an already-existing, organized anti-immunization community was fighting all vaccination programs. Lastly, in a healthcare environment severely affected by malpractice litigation, the risk of downstream liability and the potentially high costs of compensation to vaccination victims posed a threat to caregivers and healthcare administrators. As Gene Matthews (Georgia State University) summarized, "there were three concerns: liability, compensation, and risk assessment … these issues got mixed up with each other." This smallpox vaccination program was not coupled to overall public health development. Georges Benjamin (American Public Health Association) noted that single-minded attention to smallpox "sacrificed core public health activities." Rosner and Markowitz point to this program as an example of how federal dictates to the states were ill-fated, mismanaged, and detrimental to long-term infrastructure goals.

Public health is accomplished at the local level, but the Centers for Disease Control and Prevention (CDC) is the federal authority and

Early response. Hazardous materials experts enter the Hart Office Building, which had been closed after the discovery of an anthrax-laced letter in Senator Tom Daschle's office.

CREDIT: STEPHEN JAFFE/AFP PHOTO/NEWSCOM

The reviewer is at the Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011–5640, USA, and the Translational Genomics Research Institute (TGen). E-mail: Paul.Keim@nau.edu

was instrumental in the national response to the events of 2001. Whereas the anthrax letter attacks occurred in only five states, the other 45 states were consumed with testing thousands of suspicious powders and letters to reassure a frightened public. In fact, the negative results from the state labs were critical in the definition of the event boundaries and allowed the CDC to focus on the real attack. In the preceding year, the CDC had implemented a nationwide laboratory system for "anthrax" testing. In the absence of this system, all samples would have been sent to Atlanta for testing in a facility already operating over capacity. Dispersed investments in infrastructure development are cited as the most important federal response to the terrorist attacks. Prior to 9/11, bioterrorism preparedness at the CDC was slowly becoming a more important activity as the budget increased incrementally.

Rosner and Markowitz provide glimpses of the conflict within the government between those devoted to bioterrorism preparedness and those skeptics unconvinced that this focus was appropriate. Many of their interviews are rife with hindsight as public officials try to rewrite history to place their activities in the best light. Conflicting interviews will allow readers to assess for themselves the CDC's actual preparedness for bioterrorism. Optimistically, the CDC's post-9/11 response to the outbreak of severe acute respiratory syndrome (SARS) was universally seen in a positive light. Whatever the CDC was before 9/11, it is clearly a very different organization today and much better prepared for public health crises.

Rosner and Markowitz weave commentary and analysis throughout the book but conclude with some basic lessons learned. First, in a crisis the available public health infrastructure makes all the difference in the quality of the local and federal response. Although timely leadership was important, it was effective only within the constraints of what the previous years' efforts had provided. Because of the unpredictability of the next crisis, public health infrastructure is the single most important way of preparing the nation. Second, the authors argue for a redefinition of public health to be more comprehensive and to include the mental health of the population. In addition to the traditional concern with the physical well-being of the population, social and economic health need to be included in the response to crises. Third, they observed that the failure to communicate honestly to the public, even if officials have good intentions to calm a chaotic situation, will lead to the subsequent mistrust of all communication. Lastly, the authors recommend that clear lines of authority need to be established in a crisis. Local authority need not be usurped, but decisive leadership, perhaps from the federal level, is critical.

Rosner and Markowitz provide a well-researched account that should have an impact on the implementation of future public health policy. Their extensive interviews and use of public statements offer readers the opportunity to assess their research and to judge their analysis and commentary. The first-person reports of the chaos of the moment, especially in New York, will enlighten the naïve and invoke harsh memories for readers who more intimately lived through the events. So, the question remains: Are we ready? Although the authors advocate one path forward, we should never expect that the struggle to improve public health will be complete or finalized. Preparedness is an ongoing and consuming endeavor.

10.1126/science.1142469

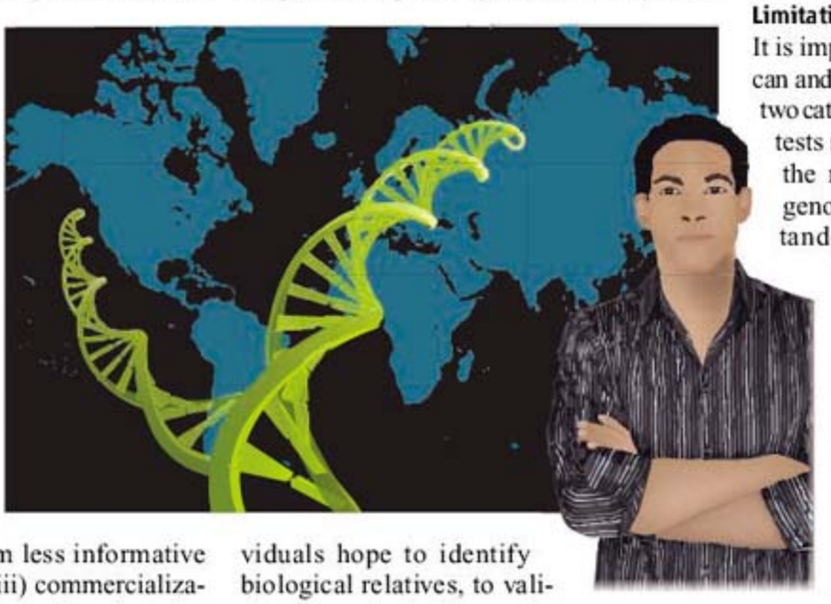
GENETICS

The Science and Business of Genetic Ancestry Testing

Commercially available tests of genetic ancestry have significant scientific limitations, but are serious matters for many test-takers.

Deborah A. Bolnick,[1]* Duana Fullwiley,[2] Troy Duster,[3,4] Richard S. Cooper,[5] Joan H. Fujimura,[6] Jonathan Kahn,[7] Jay S. Kaufman,[8] Jonathan Marks,[9] Ann Morning,[3] Alondra Nelson,[10] Pilar Ossorio,[11] Jenny Reardon,[12] Susan M. Reverby,[13] Kimberly TallBear[14,15]

At least two dozen companies now market "genetic ancestry tests" to help consumers reconstruct their family histories and determine the geographic origins of their ancestors. More than 460,000 people have purchased these tests over the past 6 years (*1*), and public interest is still skyrocketing (*1*–*4*). Some scientists support this enterprise because it makes genetics accessible and relevant; others view it with indifference, seeing the tests as merely "recreational." However, both scientists and consumers should approach genetic ancestry testing with caution because (i) the tests can have a profound impact on individuals and communities, (ii) the assumptions and limitations of these tests make them less informative than many realize, and (iii) commercialization has led to misleading practices that reinforce misconceptions.



CREDIT: N. KEMITYAGALA/SCIENCE

The Impact of "Recreational Genetics"

Although genetic ancestry testing is often described as "recreational genetics," many consumers do not take these tests lightly. Each test costs $100 to $900, and consumers often have deep personal reasons for purchasing these products. Many individuals hope to identify biological relatives, to validate genealogical records, and to fill in gaps in family histories. Others are searching for a connection to specific groups or places in Eurasia and Africa. This search for a "homeland" is particularly poignant for many African-Americans, who hope to recapture a history stolen by slavery. Others seek a more nuanced picture of their genetic backgrounds than the black-and-white dichotomy that dominates U.S. racial thinking.

Genetic ancestry testing also has serious consequences. Test-takers may reshape their personal identities, and they may suffer emotional distress if test results are unexpected or undesired (*5*). Test-takers may also change how they report their race or ethnicity on governmental forms, college or job applications, and medical questionnaires (*6*). This could make it more difficult to track the social experiences and effects of race and racism (*6*). Genetic ancestry testing also affects broader communities: Tests have led African-Americans to visit and financially support specific African communities. Other Americans have taken the tests in hope of obtaining Native American tribal affiliation (and benefits like financial support, housing, education, health care, and affirmation of identity) or to challenge tribal membership decisions (*7*).

Limitations

It is important to understand what these tests can and cannot determine. Most tests fall into two categories. Mitochondrial DNA (mtDNA) tests sequence the hypervariable region of the maternally inherited mitochondrial genome. Y-chromosome tests analyze short tandem repeats and/or single nucleotide polymorphisms (SNPs) in the paternally inherited Y chromosome. In both cases, the test-taker's haplotype (set of linked alleles) is determined and compared with haplotypes from other sampled individuals. These comparisons can identify related individuals who share a common maternal or paternal ancestor, as well as locations where the test-taker's haplotype is found today. However, each test examines less than 1% of the test-taker's DNA and sheds light on only one ancestor each generation (*8*). A third type of test (DNAPrint's AncestryByDNA test) attempts to provide a better measure of overall ancestry by using 175 autosomal markers (inherited from both parents) to estimate an individual's "biogeographical ancestry."

Although companies acknowledge that mtDNA and Y-chromosome tests provide no information about most of a test-taker's ancestors, more important limitations to all three types of genetic ancestry tests are often less obvious. For example, genetic ancestry testing can identify some of the groups and locations around the world where a test-taker's haplotype or autosomal markers are found, but it is unlikely to identify all of them. Such inferences depend on the samples in a company's database, and even databases with 10,000 to 20,000 samples may fail to capture the full array of human genetic diversity in a particular population or region.

[1]Department of Anthropology, University of Texas, Austin, TX 78712, USA. [2]Departments of Anthropology and African and African-American Studies, Harvard University, Cambridge, MA; [3]Department of Sociology, New York University, New York, NY; [4]Department of Sociology, University of California, Berkeley, CA; [5]Department of Preventive Medicine and Epidemiology, Loyola University Chicago Stritch School of Medicine, Maywood, IL; [6]Department of Sociology, University of Wisconsin, Madison, WI; [7]Hamline University School of Law, St. Paul, MN; [8]Department of Epidemiology, University of North Carolina School of Public Health, Chapel Hill, NC; [9]Department of Anthropology, University of North Carolina, Charlotte, NC; [10]Departments of Sociology and African American Studies, Yale University, New Haven, CT; [11]University of Wisconsin Law School, Madison, WI; [12]Department of Sociology, University of California, Santa Cruz, CA; [13]Department of Women's Studies, Wellesley College, Wellesley, MA; [14]Department of American Indian Studies, Arizona State University, Tempe, AZ; [15]Department of Environmental Science, Policy and Management, and Rhetoric, University of California, Berkeley, CA; USA.

*Author for correspondence. E-mail: deborah.bolnick@mail.utexas.edu

Another problem is that questionable scientific assumptions are sometimes made when companies report results of a genetic ancestry test. For instance, when an allele or haplotype is most common in one population, companies often assume it to be diagnostic of that population. This can be problematic because high genetic diversity exists within populations and gene flow occurs between populations. Very few alleles are therefore diagnostic of membership in a specific population (*9*), but companies sometimes fail to mention that an allele could have been inherited from a population in which it is less common. Consequently, many consumers do not realize that the tests are probabilistic and can reach incorrect conclusions.

Consumers often purchase these tests to learn about their race or ethnicity, but there is no clear-cut connection between an individual's DNA and his or her racial or ethnic affiliation. Worldwide patterns of human genetic diversity are weakly correlated with racial and ethnic categories because both are partially correlated with geography (*9*). Current understandings of race and ethnicity reflect more than genetic relatedness, though, having been defined in particular sociohistorical contexts (i.e., European and American colonialism). In addition, social relationships and life experiences have been as important as biological ancestry in shaping individual identity and group membership.

Many genetic ancestry tests also claim to tell consumers where their ancestral lineage originated and the social group to which their ancestors belonged. However, present-day patterns of residence are rarely identical to what existed in the past, and social groups have changed over time, in name and composition (*10*). Databases of present-day samples may therefore provide false leads.

Finally, even though there is little evidence that four biologically discrete groups of humans ever existed (*9*), the AncestryByDNA test creates the appearance of genetically distinct populations by relying on "ancestry informative markers" (AIMs). AIMs are SNPs or other markers that show relatively large (30 to 50%) frequency differences between population samples. The AncestryByDNA test examines AIMs selected to differentiate between four "parental" populations (Africans, Europeans, East Asians, and Native Americans). However, these AIMs are not found in all peoples who would be classed together as a given "parental" population. The AIMs that characterize "Africans," for example, were chosen on the basis of a sample of West Africans. Dark-skinned East Africans might be omitted from the AIMs reference panel of "Africans" because they exhibit different gene variants (*11*–*13*). Furthermore, some of the most "informative" AIMs involve loci that have undergone strong selection (*14*), which makes it unclear whether these markers indicate shared ancestry or parallel selective pressures (such as similar environmental exposures in different geographic regions) or both.

The problems described here are likely responsible for the most paradoxical results of this test. For instance, the AncestryByDNA test suggests that most people from the Middle East, India, and the Mediterranean region of Europe have Native American ancestry (*15*). Because no archaeological, genetic, or historical evidence supports this suggestion, the test probably considers some markers to be diagnostic of Native American ancestry when, in fact, they are not.

Thus, these tests should not be seen as determining the race or ethnicity of a test-taker. They cannot pinpoint the place of origin or social affiliation of even one ancestor with exact certainty. Although wider sampling and technological advancements may help (*16*), many of the tests' problems will remain.

Effects of Commercialization

Although it is important for consumers to understand the limitations of genetic ancestry testing and the complex relation between DNA, race, and identity, these complexities are not always made clear. Web sites of many companies state that race is not genetically determined, but the tests nevertheless promote the popular understanding that race is rooted in one's DNA (*17*)—rather than being an artifact of sampling strategies, contrasting geographical extremes, and the imposition of qualitative boundaries on human variation. Because race has such profound social, political, and economic consequences, we should be wary of allowing the concept to be redefined in a way that obscures its historical roots and disconnects it from its cultural and socioeconomic context.

It is unlikely that companies (and the associated scientists) deliberately choose to mislead consumers or misrepresent science. However, market pressures can lead to conflicts of interest, and data may be interpreted differently when financial incentives exist. For scientists, these incentives include paid consultancies, patent rights, licensing agreements, stock options, direct stock grants, corporate board memberships, scientific advisory board memberships, media attention, lecture fees, and/or research support. Because scientific pronouncements carry immense weight in our society, claims must be carefully evaluated when scientists have a financial stake in them. Unfortunately, peer-review is difficult here, because most companies maintain proprietary databases.

As consumers realize that they have been sold a family history that may not be accurate, public attitudes toward genetic research could change. Support for molecular and anthropological genetics might decrease, and historically disadvantaged communities might increase their distrust of the scientific establishment (*18*). These tests may also come up in medical settings: Many consumers are aware of the well-publicized association between ancestry and disease, and patients may ask doctors to take their ancestry tests into consideration when making medical decisions. Doctors should be cautious when considering such results (*19*).

We must weigh the risks and benefits of genetic ancestry testing, and as we do so, the scientific community must break its silence and make clear the limitations and potential dangers. Just as the American Society of Human Genetics recently published a series of recommendations regarding direct-to-consumer genetic tests that make health-related claims (*20*), we encourage ASHG and other professional genetic and anthropological associations to develop policy statements regarding genetic ancestry testing.

References and Notes

1. H. Wolinsky, *EMBO Rep.* **7**, 1072 (2006).
2. J. Simons, *Fortune* **155**, 39 (2007).
3. Thirteen/WNET New York, *African American Lives*, "Episode 2: The Promise of Freedom," press release (27 July 2007).
4. P. Harris, *Observer* [London], 15 July 2007, p. 22.
5. *Motherland*, "A Genetic Journey" (Takeaway Media Productions, London, 2003).
6. A. Harmon, *New York Times*, 12 April 2006, p. A1.
7. B. Hoerner, *Wired* 13 (2005).
8. A. Yang, *Chance* **20**, 32-39 (2007).
9. K. Weiss, M. Fullerton, *Evol. Anthropol.* **14**, 165 (2005).
10. C. Rotimi, *Dev. World Bioethics* **3**, 151–158 (2003).
11. S. Tishkoff *et al.*, *Nature Genet.* **39**, 31–40 (2006).
12. A. Mourant, A. Kopec, K. Domaniewska-Sobczak, *The Distribution of the Human Blood Groups and Other Polymorphisms* (Oxford Univ. Press, London, 1976).
13. M. Hamblin, A. Di Rienzo, *Am. J. Hum. Genet.* **66**, 1669–1679 (2002).
14. J. Akey *et al.*, *Genome Biol.* **12**, 1805–1814 (2002).
15. www.ancestrybydna.com/welcome/productsandservices/ancestrybydna/ethnicities.
16. M. Shriver, R. Kittles, *Nature Rev. Genet.* **5**, 611 (2004).
17. DNAPrint, Frequently asked questions, no. 1, www.ancestrybydna.com/welcome/faq/#q1.
18. J. Reardon, *Race to the Finish: Identity and Governance in an Age of Genomics* (Princeton Univ. Press, Princeton, NJ, 2004).
19. In contexts such as gene mapping and genomewide associations, genetic ancestry information can protect against confounding by population stratification or provide evidence of the population origin of specific susceptibility alleles (*21*). These applications are much narrower than determination of individual ancestry.
20. K. Hudson *et al.*, *Am. J. Hum. Genet.* **81**, 635 (2007).
21. M. Enoch *et al.*, *J. Psychopharmacol.* **20**, 19 (2006).

10.1126/science.1150098

PLANETARY SCIENCE

Sampling the Sun

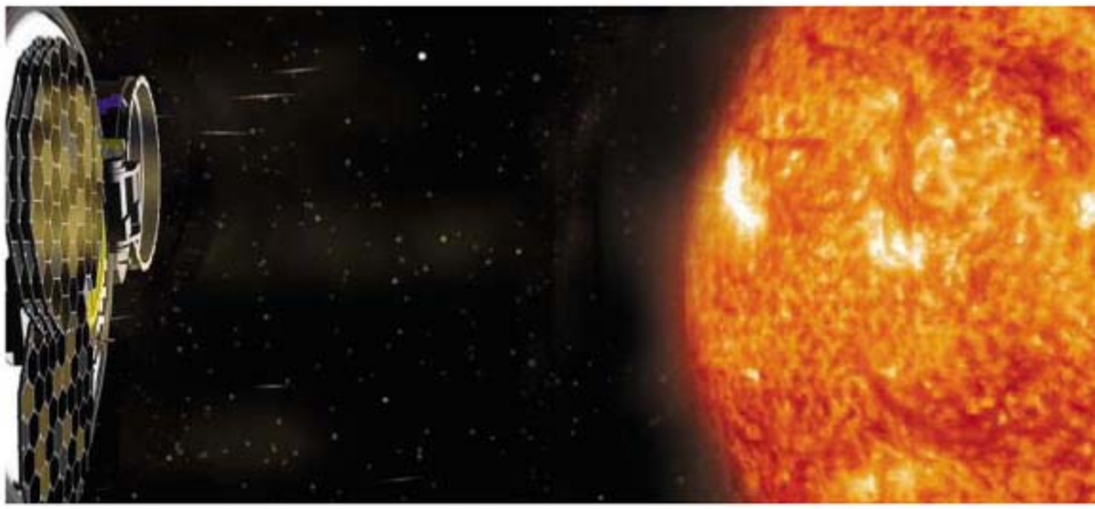
Kurt Marti

The composition of solar wind particles collected by the Genesis mission will fill gaps in our understanding of how the Sun and solar system formed.

For several years, astronomers and cosmochemists have been moving toward a scenario in which the Sun did not form as an isolated star, but as a member of a cluster in a dense molecular cloud that fostered high-mass star formation. We have known for some time that the abundances of elements in the Sun are generally consistent with those observed in primitive carbonaceous meteorites (except for volatiles). Now, on page 433 of this issue, Meshik *et al.* (*1*) report direct data on the isotopic composition of the Sun from samples of solar material collected by the NASA Genesis mission launched in 2001. Such data will help resolve the questions of how the solar system formed and in what type of environment.

A few elements in the Sun (such as the noble gases) were first studied in components of meteorites that were exposed to the solar wind before rocks formed on asteroidal surfaces, as well as in lunar soils returned by the Apollo missions. These soil grains were found to contain gases embedded in their surface layers (at depths less than 200 nm), consistent with stopping ranges of solar wind particles. This information was somewhat confusing, as discussed by Grimberg *et al.* (*2*), who reported data from stepwise etching analyses of foils recovered from the Genesis mission. These collecting foils, into which the solar particles slammed, show that solar wind plasma has one well-defined neon isotopic signature, whereas the surface layers of lunar soils showed two different signatures.

Recent research has suggested that isotopic abundances in the Sun and in other objects of the solar system may not be entirely uniform. Radiation from an early active Sun, galactic gamma and particle radiation, or catastrophic events such as mass ejection or supernova events of neighboring stars in the forming star cluster may have altered isotopic abundances in different locations. Such violent events may have injected both radioactive and stable nuclides and dust into the evolving solar nebula, possibly challenging some of the assumptions currently made for the distribution of radioactive parent elements that are necessary for deducing the earliest history of the solar system (*3*). Such injections may also account for some of the observed variations in isotopic abundances in elements such as oxygen (*4*) in different regions of the solar nebula. Researchers have found well-preserved presolar materials embedded in primitive carbonaceous chondrites, and these provide extensive records on the history of galactic element synthesis [for reviews see (*5*)].



Studying the solar wind. The Genesis spacecraft sampled particles of various energies emitted by the million-degree corona of the Sun (shown in the light of ionized helium). The image is adapted from data from the Solar and Heliospheric Observatory.

Meshik *et al.* report precise isotopic data of the noble gases neon and argon in foils of solar wind collectors carried by the Genesis spacecraft and recovered after its crash-landing. The gases were collected during specific time periods in which the solar wind had different speeds, and therefore the data sampled different solar processes and activities. Although solar wind collection experiments were conducted on the Moon during the Apollo missions (*6*), the high-precision isotope ratios from Genesis are averaged over much longer collection times than were the Apollo ratios. The solar wind isotopic signatures in foils of collectors on the Moon and on Genesis show no significant changes, although Ne/Ar abundance ratios vary by more than 30%.

An important message in the Genesis data is that isotope ratios in the high-speed wind stream (from coronal holes) and in low-speed wind, as well as in winds during coronal mass ejections, are all consistent and show no evidence for significant isotope fractionations. This will help to revise solar models and mechanisms of transport from sources in magnetic structures of the chromosphere and from transition zones to the corona, which are generally considered to be responsible for observed elemental fractionations. Detailed analyses of possible isotopic fractionations between the regimes of solar wind plasma and the solar convective surface are essential for the selection of solar data as the standards in the evaluation of solar system isotopic structures.

Grimberg *et al.* (*2*) reported data from another Genesis foil and showed that the depth-dependent concentration of neon is consistent with a single solar component, and Meshik *et al.* now document single solar isotopic signatures of neon and argon. However, both compositions differ from those observed in the terrestrial and martian atmospheres and also from isotopic abundance data observed in meteorites. The solar isotopic abundances now provide a new set of references for the interpretation of observed isotopic data in solar system matter. No isotopic references currently exist for some of the most abundant elements in the Sun, such as oxygen and nitrogen. Solar wind nitrogen embedded in the top surface layer of a lunar rock over the past 2 million years was reported to contain 3.8% more ^{15}N than the nitrogen in the terrestrial atmosphere (*7*). Because erosional processes on the Moon apparently affected solar neon isotope ratios, we should not be surprised at modifications in the nitrogen data.

Fortunately, a number of foils have survived

The author is in the Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093, USA. E-mail: kmarti@ucsd.edu

CREDIT: DRAWING OF GENESIS COLLECTOR, NASA; SOLAR IMAGE, SOHO-EIT CONSORTIUM

(not without degradation) the crash-landing of the Genesis instrument. Planetary and solar scientists are awaiting new information on the isotopic abundances of other elements collected by Genesis foils. Because solar isotopic signatures have been inferred only indirectly from abundance data in meteorites, these new solar reference data are in great demand for proper interpretations of observations on planets and in meteorites. They may help to fill some of the gaps in our understanding of how objects in the solar system formed and evolved.

References

1. A. Meshik *et al.*, *Science* **318**, 433 (2007).
2. A. Grimberg *et al.*, *Science* **314**, 1133 (2006).
3. M. Bizzarro *et al.*, *Science* **316**, 1178 (2007).
4. N. Sakamoto *et al.*, *Science* **317**, 231 (2007); published online 13 June 2007 (10.1126/science.1142021).
5. D. S. Lauretta, H. Y. McSween, *Meteorites and the Early Solar System II* (Univ. of Arizona Press, Tucson, AZ, 2006).
6. J. Geiss *et al.*, *Space Sci. Rev.* **110**, 307 (2004).
7. J. S. Kim, Y. Kim, K. Marti, J. F. Kerridge, *Nature* **375**, 383 (1995).

10.1126/science.1149379

GEOCHEMISTRY

A New Twist for Mercury

In some light-driven reactions, mercury's reactivity depends on its isotopic mass in unexpected ways, possibly allowing this element to be tracked in the environment.

Carl Lamborg

According to the biogeochemist William Fitzgerald, studying mercury is like being "on the trail of a silvery comet whose path is full of surprising twists and turns" (*1*). This volatile and mutable element has offered up yet another twist, as reported by Bergquist and Blum on page 417 of this issue (*2*).

Based on kinetic effects, heavier isotopes should be less reactive in proportion to the square root of the mass of the atom or molecule involved in the reaction. Yet, Bergquist and Blum found that ^{199}Hg and ^{201}Hg did not conform to this mass-dependent behavior (see the figure). Previously, such "mass-independent fractionation" (MIF) had only been documented for oxygen and sulfur during photochemical reactions involving ultraviolet radiation (*3*).

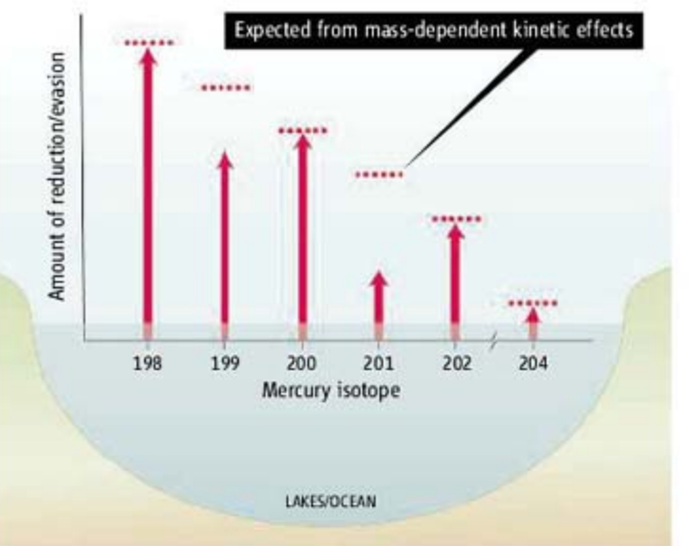
This finding offers a potentially powerful new tool for understanding the cycling of mercury in the environment. Because of its unusual volatility in the elemental state, mercury is easily exchanged between water and air and between land and air, resulting in global dispersion through the atmosphere (*4*). The process starts with the reduction of Hg^{2+} to Hg^0 vapor by biotic or abiotic reactions, resulting in supersaturation in surface waters of lakes and the ocean or high concentrations in soil interstices. The volatile Hg^0 then spends a few months to a few years in the atmosphere (*5*). Oxidation by various radical species leads to the formation of the much less volatile Hg^{2+}, which is rapidly removed from the air, closing this loop in the overall mercury cycle.

These fluxes, particularly from water to air, are difficult to measure directly. The findings of Bergquist and Blum could change that, because the initial Hg^{2+} reduction step may be largely driven by ultraviolet radiation, imprinting an MIF signal on the mercury isotope distribution. The authors also found a MIF signal in the light-driven demethylation of monomethylmercury (MeHg), the form of mercury that accumulates in biota. Thus, the magnitude of MIF signals in mercury isotope distributions in natural samples should be related to the impact of Hg^{2+} and MeHg photoreduction, because the product (Hg^0) escapes the system by water-to-air exchange. Bergquist and Blum offer an initial example: Using their laboratory-determined fractionation factor, they suggest that the MIF signal in fish can be used as a record of the amount of mercury lost from a lake or the ocean as a result of photoreduction and water-air exchange.

This report of MIF of mercury isotopes is not the first for an environmental sample, nor should it have been unexpected in hindsight. As the authors point out, ultraviolet radiation has previously been used to separate mercury isotopes under laboratory- and production-scale conditions, as part of nuclear weapons research. Jackson *et al.* previously reported MIF signals in aquatic animals and sediments from lakes (*6*). However, these are difficult measurements to make, requiring dedicated instruments and scrupulous attention to fractionation effects that arise during the analysis. Thus, results suggesting MIF from complex natural media are liable to be met with skepticism. Bergquist and Blum obtained their data during controlled laboratory experiments and for natural samples, lending enormous credibility to the finding.

Bergquist and Blum find not only mass-independent, but also mass-dependent fractionation. They and their colleagues (*7*) have documented mass-dependent fractionation in both photochemical and biological reduction of Hg^{2+}. This form of fractionation could also be very useful in tracking the biogeochemical cycling of mercury and may, in conjunction with MIF measurements, allow the impact of bacterial reduction on natural samples to be isolated and studied.

These reports are part of rapidly evolving research into heavy-element isotope fractionation made possible by advances in ultrahigh-precision isotope ratio mass spectrometry (*8*, *9*). For mercury, these studies suggest that fractionation abounds and is a fairly substantial signal. This could



Not dependent on mass. During photoreduction of Hg^{2+} and MeHg in natural waters and the subsequent water-to-air exchange of Hg^0, heavier isotopes are expected to react more slowly and to become more enriched in solution (dotted line). Bergquist and Blum show that even-numbered mercury isotopes follow this pattern, but odd-numbered isotopes instead exhibit mass-independent fractionation.

The author is in the Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA. E-mail: clamborg@whoi.edu

be enormously useful for tracking the various biogeochemical transformations in the mercury cycle. However, fractionation could be so prevalent that the signals we wish to capture are obliterated as mercury winds its way through its various environmental incarnations.

Much work remains to be done before the mass-dependent and mass-independent signals can be interpreted fully. For example, detection limitations forced Bergquist and Blum to conduct their experiments at ratios of mercury to chromophoric dissolved organic matter that were orders of magnitude higher than in natural waters. But these are surmountable problems, leading to the next twist in the trail of an irresistible geochemical mystery.

References

1. W. F. Fitzgerald, *Geochim. Cosmochim. Acta* **68**, 1961 (2004).
2. B. A. Bergquist, J. D. Blum, *Science* **318**, 417 (2007); published online 13 September 2007 (10.1126/science.1148050).
3. M. H. Thiemens, *Annu. Rev. Earth Planet. Sci.* **34**, 217 (2006).
4. W. F. Fitzgerald, C. H. Lamborg, in *Treatise on Geochemistry*, vol. 9, *Environmental Geochemistry*, B. S. Lollar, Ed. (Elsevier, New York, 2004), pp. 107–148.
5. N. E. Selin *et al.*, *J. Geophys. Res.* **112**, D02308 (2007).
6. T. A. Jackson, D. M. Whittle, M. S. Evans, D. C. G. Muir, *Geochim. Cosmochim. Acta* **70**, A284 (2006).
7. K. Kritee, J. D. Blum, M. W. Johnson, B. A. Bergquist, T. Barkay, *Environ. Sci. Technol.* **41**, 1889 (2007).
8. J. Barling, G. L. Arnold, A. D. Anbar, *Earth Planet. Sci. Lett.* **193**, 447 (2001).
9. B. L. Beard *et al.*, *Science* **285**, 1889 (1999).

10.1126/science.1149935

MOLECULAR BIOLOGY

Unlocking Cell Fate

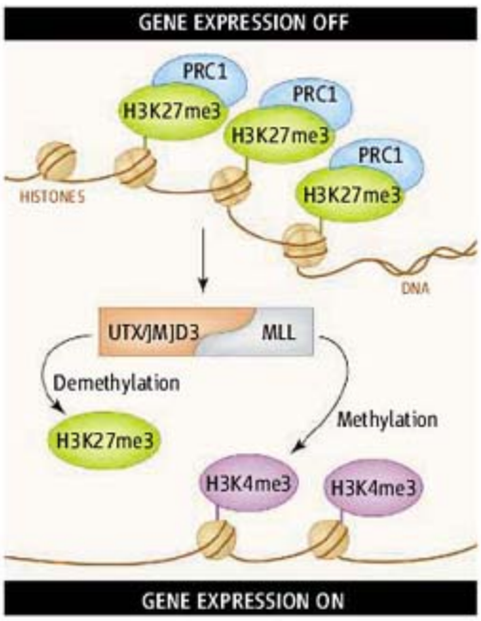
Ashley G. Rivenbark and Brian D. Strahl

Histone demethylation is associated with the activation of genes that control cellular development, differentiation, and the determination of cell fate.

Control of cell fate is a complex and poorly understood process. It is largely directed by the epigenetic regulation of gene expression—changes in gene function without changing the underlying DNA sequence. Epigenetic regulation is mediated partly through altering chromatin, the DNA and protein constituents of chromosomes. Two papers in this issue, by Chang *et al.* on page 444 (*1*) and Lee *et al.* on page 447 (*2*), advance our understanding of how epigenetic changes control cell fate and organismal development through the removal of histone methylation, a chemical modification of specific chromatin-associated proteins.

DNA is packaged within the cell's nucleus through its interaction with histone proteins (H2A, H2B, H3, and H4), which forms chromosomal regions that are either permissive or repressive for gene expression. Methylation of histones controls transcription by allowing chromosomal regions to toggle between "on" and "off" states. Moreover, this modification is reversible.

Homeotic (*Hox*) genes are fundamental in controlling embryonic development and stem cell renewal. In most differentiated cells, *Hox* genes are repressed by Polycomb group (PcG) proteins such as EZH2 (Enhancer of Zeste Homolog 2) methyltransferase, which trimethylates histone H3 at lysine 27 (H3K27me3). According to Lee *et al.*, a decrease in this specific modification during cell differentiation (*3*) is due to UTX, a demethylase specific for H3K27me3. UTX belongs to a family of enzymes that uses a Jumonji C (JmjC) domain to catalyze demethylation on lysines (*4*). Another H3K27me3 demethylase, Jumonji domain–containing 3 (JMJD3), has also recently been identified (*5*–*7*).



A genetic reprogramming "switch." UTX and JMJD3 demethylate histone H3 (H3K27me3). They also associate with the MLL complex, which methylates histone H3 (H3K4me3). This coordinated removal and addition of distinct methylation marks define an epigenetic "switch" for regulating genes that control development and cell fate.

H3K27 di- and trimethylation typically localize to the promoter region of developmentally regulated genes like the *Hox* gene clusters. Polycomb repressive complex 1 (PRC1), which contains histone H2A monoubiquitylating activity, is recruited to *Hox* genes to mediate their repression (*8*). Now, Chang *et al.*, Lee *et al.*, and the other new reports (*5*–*7*) show that the enzymes UTX and JMJD3 are recruited to *Hox* promoters, remove H3K27me3, and reverse this repression. Although UTX and JMJD3 appear to function in different contexts, and their individual or combined roles are not yet clear, their ability to control development is conclusive. For example, targeted inhibition of UTX in zebrafish and its counterpart in nematode results in posterior and gonad developmental defects, respectively (*5*, *7*). Differentiation of bone marrow progenitor cells upon cytokine stimulation is also disrupted in the absence of JMJD3 (*6*). Thus, H3K27me3 is a crucial mark in deciding cell fate.

Are there distinct roles for UTX and JMJD3 in early embryogenesis and/or in late differentiation? Both enzymes target H3K27me3, but UTX is constitutively expressed, whereas JMJD3 expression is induced in response to extracellular cues. Also, Lee *et al.* find that UTX is recruited to *Hox* genes in differentiating cells, implying that it may survey H3K27me3 globally and selectively remove H3K27me3 when given the correct developmental cues. Determining the roles of these enzymes, and other possible H3K27 demethylases, in developmental transcription cascades is a important next step.

Is removal of H3K27me3 alone enough to change cell identity or fate? The answer appears to be no. Several of the UTX/JMJD3 studies also found that loss of H3K27me3 was followed by another epigenetic change—trimethylation of histone H3 at lysine 4 (H3K4me3), which is linked to active gene transcription. Remarkably, UTX and JMJD3 are components of the MLL (Mixed Lineage Leukemia) protein complex that methylates H3K4, indicating that removal of H3K27me3

The authors are in the Department of Biochemistry and Biophysics, UNC Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. E-mail: brian_strahl@med.unc.edu

is coupled directly to formation of transcriptionally active chromatin. Thus, cell fate change involves selective recruitment of multiple histone-modifying activities that precisely alter the epigenetic landscape to activate specific developmental programs, a finding consistent with the "histone code" hypothesis (*9*). In the case of *Hox* genes, selective removal and addition of H3K27me3 and H3K4me3, respectively, define a histone code or chromatin "switch" for reprogramming genes that control cell development (see the figure). Epigenetic switches like UTX/JMJD3-MLL will no doubt occur in numerous other chromatin-mediated events (e.g., replication and DNA repair). Whether other epigenetic modifications such as DNA methylation contribute to the UTX/JMJD3-MLL switch will be interesting to determine.

The identification of histone demethylases raises the question of how epigenetic states are propagated from one cell generation to the next. If histone lysine methylation is reversible, how are epigenetic states inherited? Is it through histone arginine methylation? Until now, no arginine demethylases were known. Although enzymes convert methylarginine to a new amino acid (citrulline), this process is irreversible and changes the primary amino acid sequence (*10*, *11*). Chang *et al.* identify the first arginine demethylase, JMJD6, which selectively converts methylarginine back to arginine. This finding forces us to question whether there will be any mechanisms for epigenetic inheritance that are not reversible.

Cell fate plasticity through reprogramming of the epigenome could explain such phenomena as amphibian limb regeneration and the reprogramming of somatic cell nuclei during animal cloning, processes that reverse a terminally differentiated state. It is intriguing to speculate whether the underlying mechanism for reprogramming the epigenome in these events involves histone demethylation by UTX/JMJD3.

References

1. B. Chang, Y. Chen, Y. Zhao, R. K. Bruick, *Science* **318**, 444 (2007).
2. M. G. Lee *et al.*, *Science* **318**, 447 (2007); published online 30 August 2007 (10.1126/science.1149042).
3. L. A. Boyer *et al.*, *Nature* **441**, 349 (2006).
4. R. J. Klose, Y. Zhang, *Nat. Rev. Mol. Cell. Biol.* **8**, 307 (2007).
5. K. Agger *et al.*, *Nature*, 10.1038/nature06145 (2007).
6. F. De Santa *et al.*, *Cell*, 10.1016/j.cell.2007.08.019 (2007).
7. F. Lan *et al.*, *Nature*, 10.1038/nature06192 (2007).
8. H. Wang *et al.*, *Nature* **431**, 873 (2004).
9. B. D. Strahl, C. D. Allis, *Nature* **403**, 41 (2000).
10. G. L. Cuthbert *et al.*, *Cell* **118**, 545 (2004).
11. Y. Wang *et al.*, *Science* **306**, 279 (2004).

10.1126/science.1150321

ASTRONOMY

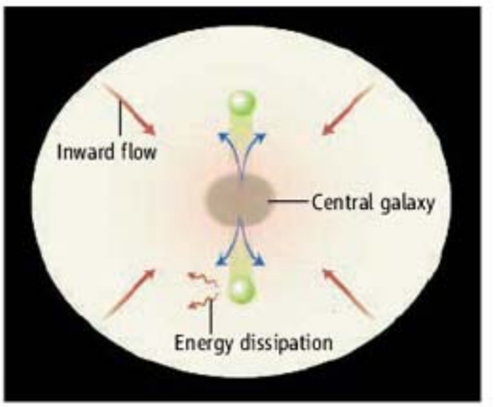
Simmering, Not Boiling

Black holes at the center of galaxy clusters can drive strong outflows of gases that limit the rate of star formation.

Christian R. Kaiser

The space between individual galaxies in clusters of galaxies is not empty. It contains hot, ionized gas, which cools rapidly by emitting x-rays. Because of these energy losses, the cooled gas should continuously flow to the centers of galaxy clusters under the influence of their enormous gravitational field. Here the gas should condense completely and form stars at a rate approaching a few thousand solar masses per year in some cases (*1*).

In fact, this enormous star formation rate is not observed (*2*), nor do clusters contain the predicted large quantities of relatively cool gas (*3*). Something is reheating the gas at a rate equivalent to about one supernova explosion every 30 years. However, supernovae within the galaxies cannot provide this energy, because they would strongly pollute the cluster gas with heavy elements (*4*), and we do not observe this. As recent work shows, more promising, "clean" heat sources are the powerful gas outflows driven by the release of gravitational energy from matter falling onto very massive black holes at the centers of galaxies embedded in the clusters. The interplay of radiative cooling and heating by outflows over cosmic time may explain the properties of current clusters and the galaxies inside them.



Central heating. The hot gas in galaxy clusters is denser toward the center than in the cluster outskirts. Radiatively cooled gas flows inward. If unchecked, this flow will strengthen, causing high rates of star formation in the central galaxy. The black hole in the central galaxy may drive a powerful gas outflow if it is accreting matter from its immediate surroundings. This inflates bubbles of hot gas (green) that can move buoyantly in the atmosphere of the cluster. The bubbles heat the cluster gas, reducing or even stopping the cooling flow. In their wakes, the bubbles lift up cold gas (blue arrows) from the central galaxy which has been enriched with elements heavier than hydrogen and helium by the stars in this galaxy.

The central regions containing black holes are called active galactic nuclei (AGN), and they produce short-lived outflows carrying enough energy to entirely remove the gas from the central region of the cluster. Although such catastrophic outflow events may play a role during the formation of galaxies in the early universe (*5*), the heating in present-day clusters is much gentler. AGN outflows inflate bubbles of extremely energetic, magnetized plasma in the cluster gas (see the figure), which we observe by their radio wave emission or through the lack of x-ray emission of the gas pushed aside (*6*).

The bubbles are much lighter than their surroundings and start to move in the gas buoyantly away from the center of the cluster (*7*). As they move, they dissipate part of their energy to the cluster gas, thereby heating it. The situation is similar to boiling water in a pot in which bubbles of steam buoyantly rise. However, the entropy of the water in the pot is highest at the bottom of the pot and decreases higher up. In galaxy clusters, the opposite applies (*8*). The heating by AGN outflows must be gentle enough to preserve the negative entropy gradient.

The buoyant motion of the bubbles also drags interstellar gas from within the host galaxy of the AGN into the cluster gas farther out. The interstellar gas is much colder than the cluster gas and rich in elements heavier than hydrogen and helium. Using the Doppler shifts of atomic emission lines, researchers have measured the velocity field of this uplift in the

The author is in the School of Physics and Astronomy, University of Southampton, Southampton SO17 1BJ UK. E-mail: crk@soton.ac.uk

wake of the bubbles and thus constructed a detailed picture of the buoyant flow in the cluster gas (*9*). Again, the buoyant motion cannot be too vigorous, as this would be inconsistent with the observed gradients in the abundances of heavy elements in the cluster gas (*10*).

The distribution of gas in clusters is remarkably similar between systems (*8*). The uniformity of clusters seems to indicate a closely balanced equilibrium between radiative cooling and AGN heating, both spatially and in time. Observations only provide us with snapshots of the cluster evolution. Therefore, it is difficult to understand how the comparatively short-lived and spatially confined AGN outflows can achieve this delicate balance.

In recent years, sophisticated numerical fluid simulations of the AGN–cluster gas interaction (*11*) have progressed rapidly to include more and more of the relevant physical processes. For example, simulations indicate that the distribution of energy throughout the clusters may be facilitated by gas motions in the cluster gas induced by their ongoing formation as nodes within the large-scale cosmic web of matter (*12*). However, it is possible that the state of the ionized and magnetized cluster gas is not well described by currently available computer simulation codes (*13*).

Another major puzzle in the field is the synchronization between the cooling gas and the AGN activity. The AGN needs to turn up the heat just at the right time; otherwise, the cluster gas would become colder than observations find. In principle, the problem looks easy to solve. The black hole in the AGN needs to accrete gas to drive an outflow. This fuel could be provided by the cooling gas in the cluster itself, and a feedback loop may be established (*14*). The problem is that the gas needs to travel several thousand light years, from the cluster all the way down to the AGN, with a size comparable to our solar system. Along the trip, it must lose all of its angular momentum in order to accrete onto the black hole, rather than to end up in a huge gas disc, rotationally supported against further contraction. It is not yet clear how the cooling gas from the cluster can be funneled to the black hole fast enough to enable a feedback loop.

Despite all of the problems listed above, AGN outflows seem set to explain the continuing presence of hot gas in galaxy clusters. It will be interesting to see how the new vast surveys planned at wavebands extending from radio to x-rays will shed light on the remaining problems through the provision of very large numbers of observed galaxy clusters. At the same time, new numerical codes and simulations will help us unravel the evolution of cluster under the influence of AGN simmering (but not boiling).

References

1. A. C. Fabian, *Annu. Rev. Astron. Astrophys.* **32**, 277 (1994).
2. B. R. McNamara, R. W. O'Connell, *Astrophys. J.* **98**, 2018 (1989).
3. B. M. Peterson *et al.*, *Astron. Astrophys.* **365**, L104 (2001).
4. K. K. S. Wu, A. C. Fabian, P. E. J. Nulsen, *Mon. Not. R. Astron. Soc.* **318**, 889 (2000).
5. A. J. Benson *et al.*, *Astrophys. J.* **599**, 38 (2003).
6. L. Birzan *et al.*, *Astrophys. J.* **607**, 800 (2004).
7. E. Churazov *et al.*, *Astrophys. J.* **554**, 261 (2001).
8. M. Donahue *et al.*, *Astrophys. J.* **643**, 730 (2006).
9. N. A. Hatch *et al.*, *Mon. Not. R. Astron. Soc.* **380**, 33 (2007).
10. J. S. Sanders, A. C. Fabian, *Mon. Not. R. Astron. Soc.* **371**, 1483 (2006).
11. C. R. Kaiser, M. Brüggen, *Nature* **418**, 301 (2001).
12. S. Heinz *et al.*, *Mon. Not. R. Astron. Soc.* **373**, L65 (2006).
13. A. A. Schekochihin *et al.*, http://arxiv.org/abs/0704.0044.
14. C. R. Kaiser, J. J. Binney, *Mon. Not. R. Astron. Soc.* **338**, 837 (2003).

10.1126/science.1148788

GENETICS

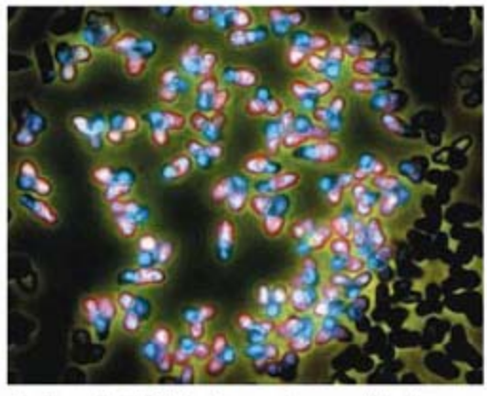
Why Genomes in Pieces?

Some microorganisms are evolutionary puzzles in that their genomes contain encrypted genes that are descrambled into gene products.

Laura F. Landweber

Microbial eukaryotes take ample detours along the route from DNA to messenger RNA (mRNA) and protein. Some of their tricks continue to erode the notion of a gene beyond its natural subdivision into functional exons and noncoding introns (*1*). Two discontinuous genetic systems described in this issue further challenge this dogma. On page 415 of this issue, Marande and Burger report a fully scrambled mitochondrial genome in *Diplonema papillatum*, a free-living relative of disease-causing trypanosomes (*2*), and on page 450, Soma *et al.* describe a set of scrambled transfer RNA (tRNA) genes in the nuclear genome of the red alga *Cyanidioschyzon merolae* (*3*). The findings are reminders that a genome sequence can be a far cry from knowledge of gene products.

Marande and Burger explode the notion of a gene with mRNA building blocks present as "modules" of ~165 base pairs, each on a separate chromosome in the mitochondria of the protist *D. papillatum*. Construction of a complete mRNA requires joining up to nine modules through a mechanism that appears distinct from known forms of RNA splicing, the processes that join exons in eukaryotic mRNA. Although split genes occur in other systems (including *Chlamydomonas*, *Euglena*, Alveolata, plants, and Diptera), rarely are the scrambled pieces "sewn" back together to create a contiguous gene or RNA. Some exceptions are gene unscrambling in ciliates (*4*) and the *bursicon* gene in mosquitoes (*5*). Trans-splicing of RNA (*6*) and even proteins (*7*) can also merge functional regions located on dispersed elements of prokaryotic (*7*) or eukaryotic genomes.



Backwards. tRNAs in *C. merolae* assemble from scrambled genes. Red plastids autofluoresce; blue color is DAPI stain (*14*).

The pathway for gene assembly in diplonemid mitochondria may provide a clue to the origin of U-insertional RNA editing, which makes a modest appearance in the report by Marande and Burger as six non–DNA-encoded uracil (U) residues that join two RNA modules. Perhaps the ancestral role of guide RNAs that direct U insertion and deletion in the related kinetoplastid protozoa was to provide a template scaffold to link modules. Such small antisense RNAs may later have gained a role in RNA editing, possibly under selective pressure to repair a region or restore a reading frame after loss or erosion of a module.

Soma *et al.* describe a new layer of tRNA processing in the red alga *C. merolae*: circularly permuted tRNAs, with the coding region

The author is in the Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544, USA. E-mail: lfl@princeton.edu

for the RNA 3′ end located upstream of the coding region for its 5′ end. Although circular permutation has been a laboratory tool for the study of RNA structure and function for years, true biological occurrences were previously known only in phage (*8*) and ciliate mitochondria (*9*). Maturation of tRNA is an elaborate RNA- and protein-driven cascade of clipping, coiffing, and adorning an initial RNA transcript (*10*). Soma *et al.* add one more decryption step to this assembly line.

Why do quirky genetic architectures emerge and persist? Some genetic systems may provide a source of evolutionary novelty. For example, module recycling or shuffling could generate new gene products without destroying the old ones or requiring duplication (*1*). So far, the gene products in *D. papillatum* seem conventional, but examination of the mitochondrial proteome may tell otherwise. Some genetic systems may be molecular fossils—neutral vestiges of the past without any special benefits. It is parsimonious to assume that the earliest organisms had split genes, for instance (*1*), but because all extant life has been evolving for the same length of time from a common ancestor, one cannot infer the preservation of ancestral genome organization without detailed mapping of ancestral and derived characters on a reliable phylogeny.

Another possible explanation for rococo genetic systems is atavism, in which some biological mechanisms revert back to an ancestral state, although presumably with modification, in a new, derived genetic background. Some of these events may appear to recapitulate features of primitive genomes, providing indirect clues as to how early genetic systems could have functioned.

There is also pure chance, a scenario that is probably slightly deleterious. Unconstrained by dogma and size, why shouldn't microbial life explore a broad range of possibilities? Protists, often reproducing asexually in the wild, would gradually accumulate small mutations and genome rearrangements that would be crippling without a mechanism to mitigate the effect. Acquisition of a new mechanism may be successful if the organism can recruit a preexisting cellular function or template for repair or rearrangement and then elaborate on the basic mechanism, leading to fixation and expansion of a complex genetic system.

Reductive evolution could account for the svelte genome size (16.5 Mb) of *C. merolae* and perhaps even some of its quirky genome architecture, if a few spandrels arose as by-products of genome compaction. This is consistent with its recent placement as a derived lineage within an outgroup of red algae (*11*). Genome reduction may lead to intrachromosome rearrangement (*12*) or overlapping genes in related protists (*13*), as either a consequence of, or adaptation to, small size. Germ-line rearrangements can also yield gene duplications, which would be trimmed back under the sword of reductive evolution. Thus, the model that Soma *et al.* propose for the origin of a permuted tRNA gene is feasible, albeit via secondary acquisition: tRNA gene duplications could emerge along with other germ-line rearrangements, and then the 5′ end of the upstream gene would be lost, as well as the 3′ end of a downstream gene, leaving the organism no choice but to exploit such a resulting permuted gene, if it can. The only option would be to rescue it by adding a few more acrobatic steps to the already-complex tRNA-processing cascade (*10*). Clearly there is a need for a suite of tRNA sequences, or better yet, comparative genomes, both closely and distantly related to *C. merolae*, to decipher the evolutionary history of its permuted tRNA genes.

Evolution is a tinkerer, and its products are not necessarily neat or elegant. Like a Rube Goldberg invention, it builds upon existing parts, embracing all their gawkiness but gradually smoothing out operations with optimization over time. The biological results are often robust systems that, in the case of protists, may not seem so at first glance.

References and Notes

1. W. Gilbert, *Nature* **271**, 501 (1978).
2. W. Marande, G. Burger, *Science* **318**, 415 (2007).
3. A. Soma *et al.*, *Science* **318**, 450 (2007).
4. W.-J. Chang, P. D. Bryson, H. Liang, M. K. Shin, L. F. Landweber, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 15149 (2005).
5. H. M. Robertson, J. A. Navik, K. K. O. Walden, H.-W. Honegger, *Genetics* **176**, 1351 (2007).
6. O. Malek, A. Brennicke, V. Knoop, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 553 (1997).
7. H. Wu, Z. Hu, X. Q. Liu, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 9226 (1998).
8. T. Pan, O. C. Uhlenbeck, *Gene* **125**, 111 (1993).
9. R. Kamikawa, Y. Inagaki, Y. Sako, *Protist* **158**, 239 (2007).
10. K. Nakanishi, O. Nureki, *Mol. Cell* **19**, 157 (2005).
11. H. S. Yoon, J. D. Hackett, C. Ciniglia, G. Pinto, D. Bhattacharya, *Mol. Biol. Evol.* **21**, 809 (2004).
12. B. Palenik *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 7705 (2007).
13. B. A. P. Williams, C. H. Slamovits, N. J. Patron, N. M. Fast, P. J. Keeling, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 10936 (2005).
14. M. Matsuzaki *et al.*, *Nature* **428**, 653 (2004).

15. Supported by NSF grant 0622112 and NIH grant GM59708. I thank C. Delwiche for discussion.

10.1126/science.1150280

CHEMISTRY

Nano-Golden Order

Robert L. Whetten and Ryan C. Price

An experimental tour de force reveals the crystal structure of a gold-thiolate nanocrystal compound and the surprising nature of the gold-sulfur bonding.

Iridescent gold nanostructures, exploited since antiquity in the medical, decorative, and theological arts, have at last begun to succumb to total structure determination, the sine qua non of the molecular sciences. On page 430 of this issue, Jadzinsky *et al.* report a breakthrough in this field: the total structure determination of a large gold-thiolate cluster (*1*). The work will help to nail down the detailed structure of self-assembled monolayers (SAMs), which are chemically modified gold electrodes with applications from sensing to nanolithography (*2*).

Atomic structures of ever larger molecules are determined by x-ray crystallography, but structural studies of nanometer-scale metal clusters (*3*) or of surfaces are still challenging. In the case of clusters, creating identical clusters can be difficult, although important exceptions have been reported (*4*). Surface diffraction studies also encounter numerous resolution-limiting problems, such as large contributions from subsurface metal layers and multiple scattering effects.

The molecules used to create SAMs on gold surfaces (see the figure, top panel) consist of an organic group (R) linked via a sulfur atom (S) to gold substrate atoms (Au). Extended SAMs on crystalline substrates are typically highly ordered, allowing the orientation of the R group to be determined, but the structure of the buried head group is often only inferred. However, structural models built on the basis of such inferences (see the figure, top panel) have been hotly contested.

Because chemical bonding is largely a local affair, one approach to attacking this nanostructure problem is to use gold nanocrystals as the metal substrate (see the figure, bottom left) (*4*, *5*), on the assumption that the gold-thiolate bond is constant at all scales and that a suitable sample will crystal-

The authors are in the School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA. E-mail: whetten@chemistry.gatech.edu

lize for total structure determination. Massive thiolate monolayer-protected gold clusters of various dimensions and with different R groups have been produced, usually from decomposition of the corresponding nonmetallic Au(I)-thiolate precursors (see the figure, bottom right). The chemical and electrical properties of these clusters usually closely resemble those of the extended SAMs. They remain exceptionally stable during size separation and analysis, stimulating claims of molecular levels of homogeneity, but until now no total structure determination has been achieved (*6*).

Enter Jadzinsky *et al.*, who report the successful isolation, crystallization, and structure determination of a cluster with 102 gold atoms and 44 RS groups (see the figure, bottom left). They accomplished this feat by using water-soluble, rigid aromatic benzene thiolates (*7*) with a carboxylate group (not shown in the figure) on the opposite side of the aromatic ring from the sulfur atom. Crystallization trials with the reaction products yielded large single crystals suitable for x-ray diffraction measurements. Given the great effort previously expended to deduce structural aspects of such clusters, it is a relief to find that the structural features of the cluster are mostly familiar (*8*), although they are combined in ways that could not have been guessed.

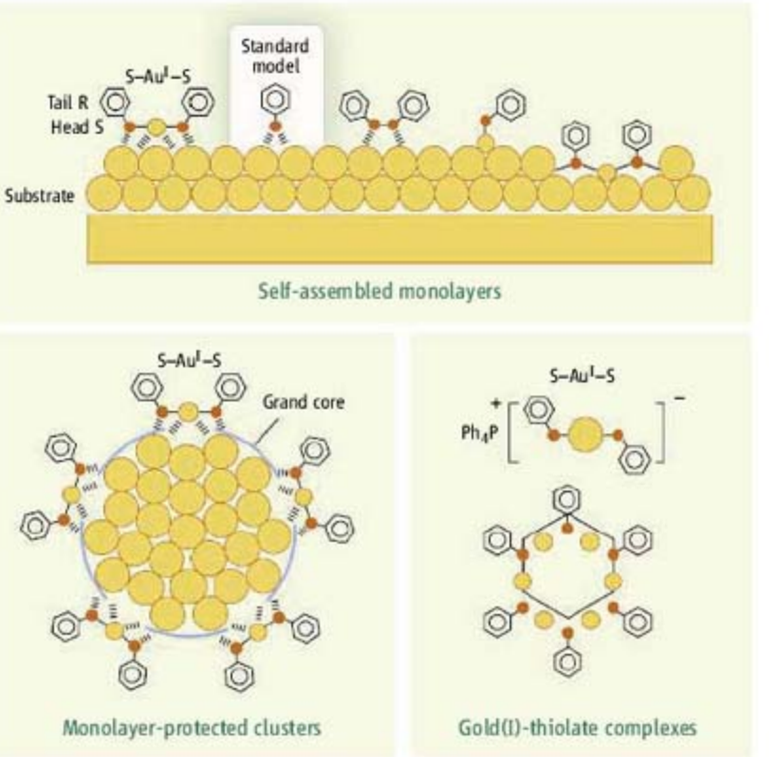
Seventy-nine of the 102 gold atoms form a truncated-decahedral cluster. This "grand core" has gold-gold bond lengths and coordination consistent with bulk metals, supported gold nanocrystals, and a prior model for gold-thiolate clusters ~1.5 nm in diameter (*9*). In an unexpected twist, the 15-atom caps at each pole are rotated with respect to the ideal compact decahedral shells, yielding denser packing and placing all 40 surface atoms of the grand core on the surface of a common sphere.

The remaining gold atoms form an outer shell that interacts strongly with the thiolate sulfur atoms. Jadzinsky *et al.* obtained a clear picture of this interaction (the "surface-chemical bond"). The results are in striking contrast to the standard model (see the figure, top), in which independent thiyl groups are attached directly to close-packed gold substrates. Rather, the binding motif exemplifies the "divide-and-protect" model (*10*), in which oligomeric aurous Au(I)-thiolate complexes interact weakly with the surface atoms of the grand core (see the figure, bottom left). Surface species of this type have analogs in known anionic complexes (see the figure, bottom right) (*11*, *12*).

The weak interactions of the thiolates with the surface atoms of the grand core are least well understood. A strongly polar, if not ionic, interaction is suggested by the distinctive anchoring of thiolate sulfur atoms in formally anionic complexes (*10*, *11*) to single substrate gold atoms in a correspondingly positive core. The close approach of Au(I) atoms within the $Au(SR)_2$ adsorbates to the surface atoms of the grand core is consistent with relativistically enhanced dispersion interactions (*13*). To gain additional insight, thermodynamic measurements and first-principles theory are crucially needed.

It is intriguing that the cluster's composition—with 102 gold atoms and 44 thiolates—suggests an additional electronic origin for its stability. In metal-cluster physics, electronic rules of cluster stability are well established. According to these rules, filled concentric angular-momentum shells of electrons confer electronic structural stability to a cluster. One such filled shell requires 58 electrons to occupy 29 delocalized orbitals. This number is achieved exactly if each of the 102 gold atoms donates one electron to delocalized orbitals, and if each of the 44 thiyl radicals ($RS^{\cdot}$) formally takes one electron into a localized orbital. Electronic rules of cluster stability have been used widely in gas-phase elemental clusters, but only rarely in molecular cluster chemistry (*14*); the current results suggest that they should perhaps be applied more widely.

It is fortuitous that a simple procedure (reductive degradation of oligomeric precursors) yields a self-assembled, discrete compound (*15*) that contains structural organization consistent with known precedents and classification (*11*). The known properties of nanoscale clusters can now be rationalized in terms of atomic ordering. Do structures of this type hold generally for flatter clusters and extended SAMs, for smaller clusters with more highly curved surfaces, for non-aromatic R groups, and for diverse gold-plated and gold-alloy nanostructures? If so, then there is hardly a published interpretation (for example, of electron transfer, capacitance, or density) that will not be in need of revision or reinterpretation.



How do thiolates bind to gold? Several binding modes for self-assembled monolayers have been suggested (**top**). Gold-sulfur binding modes in the 102-gold-atom cluster reported by Jadzinsky *et al.* (*1*) (**bottom left**) may reveal the structure of self-assembled monolayers. Gold(I)-thiolate compounds have also been structurally characterized (**bottom right**). In all panels, gold atoms are depicted as yellow circles, sulfur as orange circles; the hexagons denote a phenyl ($-C_6H_5$) aromatic hydrocarbon.

References

1. P. D. Jadzinsky, G. Calero, C. J. Ackerson, D. A. Bushnell, R. D. Kornberg, *Science* **318**, 430 (2007).
2. J. C. Love *et al.*, *Chem. Rev.* **105**, 1105 (2005).
3. S. J. L. Billinge, I. Levin, *Science* **316**, 561 (2007).
4. E. G. Mednikov, M. C. Jewell, L. F. Dahl, *J. Am. Chem. Soc.* **129**, 11619 (2007).
5. A. C. Templeton, W. P. Wuelfing, R. W. Murray, *Acc. Chem. Res.* **33**, 27 (2000).
6. R. L. Whetten *et al.*, *Adv. Mater.* **8**, 428 (1996).
7. R. C. Price, R. L. Whetten, *J. Am. Chem. Soc.* **127**, 13750 (2005).
8. H. Schmidbaur, Ed., *Gold: Progress in Chemistry, Biochemistry, and Technology* (Wiley, New York, 1999), chapters 10, 15, and 20.
9. B. G. Bagley, *Nature* **225**, 1040 (1970).
10. H. Hakkinen, M. Walter, H. Grönbeck, *J. Phys. Chem. B* **110**, 9927 (2006).
11. J. M. Waters, *Acta Crystallogr.* **41**, 862 (1985).
12. J. Strahle, *Chem. Ber.* **124**, 2161 (1991).
13. P. Pyykkö, *Angew. Chem. Int. Ed.* **43**, 4412 (2004).
14. P. Ball, *New Scientist* (16 April 2005), p. 30.
15. R. F. Service, P. Szuromi, J. Uppenbrink, Eds., special issue on Supramolecular Chemistry and Self-Assembly *Science* **295** (29 March 2002).

10.1126/science.1150176

Mathematics and Complex Systems

Richard Foote

Contemporary researchers strive to understand complex physical phenomena that involve many constituents, may be influenced by numerous forces, and may exhibit unexpected or emergent behavior. Often such "complex systems" are macroscopic manifestations of other systems that exhibit their own complex behavior and obey more elemental laws. This article proposes that areas of mathematics, even ones based on simple axiomatic foundations, have discernible layers, entirely unexpected "macroscopic" outcomes, and both mathematical and physical ramifications profoundly beyond their historical beginnings. In a larger sense, the study of mathematics itself, which is increasingly surpassing the capacity of researchers to verify "by hand," may be the ultimate complex system.

Since antiquity, humankind has pondered and debated the "unreasonable effectiveness of mathematics" in its apparent ability to explain physical phenomena, an enigma elucidated by Wigner [(*1*), p. 1]: "Mathematical concepts turn up in entirely unexpected connections. Moreover, they often permit an unexpectedly close and accurate description of the phenomena in these connections." In recent years the scientific community has coined the rubric "complex system" to describe phenomena, structures, aggregates, organisms, or problems that share some common themes: (i) They are inherently complicated or intricate, in that they have factors such as the number of parameters affecting the system or the rules governing interactions of components of the system; (ii) they are rarely completely deterministic, and state parameters or measurement data may only be known in terms of probabilities; (iii) mathematical models of the system are usually complex and involve nonlinear, ill-posed, or chaotic behavior; and (iv) the systems are predisposed to unexpected outcomes (so-called "emergent behavior"). Familiar examples include weather systems, biological and chemical systems, social networks, transportation and engineering infrastructure systems, and the Internet (*2*–*4*).

In these examples, the complex systems are physical phenomena that researchers attempt to model mathematically. But areas of mathematics itself may be viewed as complex systems exhibiting many of the characteristics of the physical structures, including discernible "layers" closely analogous to microscopic or macroscopic strata in physics, biology, and other sciences. As we struggle to better understand and systematize precise notions embodying physical complex systems, and ultimately to fathom the potential for mathematics to model these, a closer look at "abstract" complex mathematical systems is in order. We may then hope to glean from the theoretical setting a more rigorous grasp of some mathematical underpinnings that characterize complex systems in the natural world, and thereby make a chink in the Wigner conundrum.

Department of Mathematics and Statistics, University of Vermont, Burlington, VT 05405, USA. E-mail: foote@math.uvm.edu

Background from Group Theory

Some developments in finite group theory are useful illustrations of the idea that aspects of mathematics represent inherently complex systems [see (*5*) for other examples]. Finite group theory emerged from a confluence of many ideas in 18th- and 19th-century abstract algebra, most notably the general insolvability of polynomial equations by radicals as pioneered by Galois around 1830 (see below). Galois showed that each polynomial is associated to a mathematical structure called a group, and the roots of the polynomial can be written in terms of radicals involving its coefficients—generalizing the familiar formula for roots of quadratic equations—if and only if the associated group can be "factored" (in a way made precise shortly) into simple group factors that all have prime order. This succinct formulation of the cornerstone of what is now known as Galois theory is possible with historical hindsight: The axiomatic formulation of the concept of a group first appeared in 1882, and shortly thereafter it was shown that every finite group—these are the ones in Galois theory—has a "unique factorization" into simple group factors (the simple group factors need not have prime order in general, however).

Before going further, a digression into some precise concepts from abstract algebra is essential for understanding the scope of the "complex system" initiated by Galois and his cohort. A group is any set G that has an operation on pairs of elements of G satisfying some simple axioms. The operation between elements a and b of G is denoted $a * b$; note that in specific groups the operation may be addition, matrix multiplication, function composition, etc., depending on the exact nature of the elements in G. The axioms for a group are:

1. The operation is associative: $a * (b * c) = (a * b) * c$, for every a, b, c in G.

2. There is an identity, denoted by the symbol 1, such that $1 * a = a * 1 = a$, for every a in G.

3. Every element a in G has an inverse, denoted by a^{-1}, such that $a^{-1} * a = a * a^{-1} = 1$.

A group is called finite if the underlying set G contains only a finite number of elements; the order of G is the number of elements in G. Groups abound in mathematics: The most familiar examples are the set of all integers, the set of all rational numbers, or the set of all real numbers, where in each instance the operation is addition, the identity is the number zero, and the inverse of an element is its negative. Likewise, the nonzero rational or real numbers (but not the integers) form a group under the operation of multiplication, where identity is the number 1 and the inverses are reciprocals. Note that in general the group operation need not be commutative; that is, we need not have $a * b = b * a$, for every a, b in G.

Finite groups arise naturally in many contexts; the most familiar are as groups of symmetries of geometric or physical objects. A regular n-sided planar figure or n-gon has $2n$ symmetries, and these symmetries form a group under the operation *, where $a * b$ denotes "first apply symmetry b and follow that by applying symmetry a to the n-gon" (this is just function composition, as one computes the composition $f[g(x)]$ of functions f and g in calculus). Such symmetry groups are not, in general, commutative—for example, the group of six symmetries of an equilateral triangle.

A nonempty subset H of a finite group G is called a subgroup of G if it is closed under the group operation: Whenever a, b belong to the subset H, their group product $a * b$ also belongs to H (in this situation, H is a group in its own right). A subgroup H of a group G is called normal if it satisfies a "weak commutativity" rule: $aH = Ha$, for every a in G, where aH is the set of all products $a * h$, over all h in H (likewise, Ha is the set of all $h * a$).

The fundamental importance of normal subgroups, first realized by Galois, is that the whole group G may be "factored" or "collapsed" into a new group by "collapsing" the set of all the elements of H to a single point, and likewise calling each set aH a single point in this new group; the operation in this new group inherits from the original group via the rule $(aH) * (bH) = (a * b)H$, where a and b are arbitrary elements of G. The resulting new group is called the quotient group of G by H and is denoted as G/H. This quotient group has order equal to the order of G divided by the order of H, and there is a natural "projection map" from the original group to the quotient group that is compatible with both their operations.

We may view the group G as "covering" the group G/H by n sheets, where H has n elements. Figure 1 illustrates this when G is the group of real numbers (operation addition), $G/H = U$ is the unit circle in the complex plane (which is a group under multiplication of complex numbers), and the projection map is $\varphi: \theta \rightarrow \exp(i\theta)$ (where H is the subgroup of G consisting of integer multiples of 2π, and n is infinite).

It is reasonable to view a group G possessing a normal subgroup H as "factored" into two smaller groups, H and G/H, and many global properties of the original group might be inferred from properties of these "factors." Indeed, this

process may be continued in a finite group by factoring both H and G/H in turn by their normal subgroups, and so on inductively until one obtains a sequence of factors that cannot be broken down further. These irreducible factors, the "primes" of group theory, are the simple groups: A group is called simple if it has at least two elements and its only normal subgroups are the obvious ones—namely G itself and the subgroup of order 1 consisting of only the identity element.

The above process illustrates that every finite group has a "factorization" into simple groups. It is also quite elementary (the Jordan-Hölder Theorem) to prove that for any given G, although different routes may be taken to achieve factorizations into its simple components, the number of factors and the intrinsic type of the factors are independent of the route (*5*). In other words, every finite group possesses an essentially unique factorization into simple factors, in much the same way as an integer factors uniquely into primes. The simple groups are thus the primes or "atoms" of finite group theory.

Galois exploited this "atomic" decomposition in an astounding way. It is quite elementary to show that finite groups whose orders are prime numbers are always simple (there is essentially a unique group of prime order p for each prime number p). Galois proved that to each polynomial of degree n there is associated a finite group in such a way that the roots of the polynomial may be expressed in terms of radical expressions involving its coefficients (as is the case for quadratics) if and only if the simple factors for its group all have prime order. He went on to show that there are polynomials of degree 5 (or any higher degree) whose groups contain simple factors that are not of prime order; this is the famous "insolvability of the quintic" theorem of Galois and Abel. This theorem was the first inkling of the complexity of this branch of mathematics: The existence of a simple group containing 60 elements—which is the smallest simple group of nonprime order—is the obstruction to there being an algebraic formula for the roots of a quintic polynomial.

This and many other properties of groups fall into the realm of elementary abstract algebra normally covered in a substantial college-level introduction [see (*6*)]. One may argue that this elementary, axiomatic field of mathematics, although perhaps complicated and mathematically deep, does not constitute a true complex system in the current scientific parlance. Indeed, one might contend that finite group theory, being completely deterministic, cannot beget emergent behavior, but perhaps can only produce "unexpected" results (or, to a Platonist, unexpected theorems may be uncovered). The import of this case study, however, is to reexamine some of the fundamental premises of what attributes truly are the hallmarks of complex systems. After all, it is futile to try to predict truly unpredictable or random systems; rather, the aim of complex systems is to explain and understand the local ("atomic") workings and rules of a system, and then to make global connections and inferences based on this knowledge. Finite group theory may help to serve as a paradigm for this thesis. Pursuing our historical explication, we discern at least three "layers" or "jumps in complexity," even within this seemingly elementary discrete realm.

The Classification of Finite Simple Groups

In the 1880s the quest began to classify finite simple groups, in the sense of listing them all in "families" that enjoy common structural properties. For example, given any natural number $n > 1$ and any finite field F (such as the integers modulo p, for any prime p), the set of all $n \times n$ matrices of determinant 1 with entries from F becomes a simple group after we factor out the scalar matrices (except in a handful of small cases). This collection of (linear) simple groups constitutes one family, indexed by the two parameters n and F [see (*5*) for other examples and precise definitions]. By the early 1960s, 18 infinite families of simple groups were known. In addition, there were five simple groups not belonging to any of these families, the five Mathieu groups; these were named "sporadic simple groups." At that time, however, there was little progress toward showing that this list was complete.

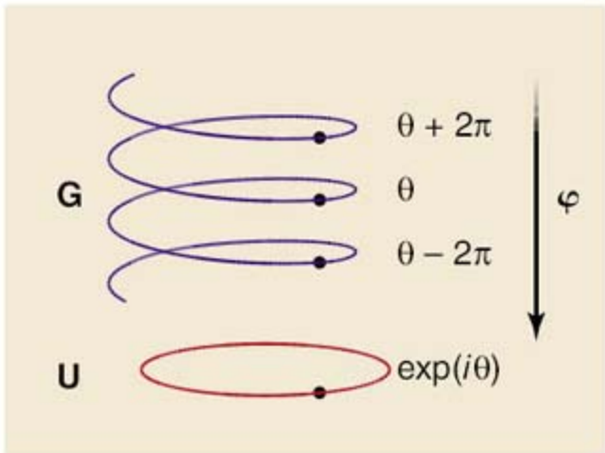


Fig. 1. Projection map φ from the real numbers G onto the circle group U.

A breakthrough to the next level of complexity occurred in 1962 when Feit and Thompson proved the celebrated Odd Order Theorem (*7*): The only simple groups containing an odd number of elements are those of prime order. This proof, which occupies an entire journal issue, consists of 255 pages of intricate, delicate, and ingenious mathematics; considerable efforts to simplify it since then have met with only modest success. This exemplifies a not uncommon phenomenon in mathematics: A theorem with a strikingly elementary formulation may—initially at least—necessitate a very complicated proof. The Feit-Thompson Theorem, although a fully anticipated result, nonetheless spawned the first "quantum jump" in technical virtuosity that practitioners would need in order to surmount problems in this arena. Numerous proofs more than 100 pages in length sprang forth, including Thompson's successor to the Odd Order Theorem, the N-group Theorem (*8*), requiring more than 400 pages of proof.

In the early 1960s, a number of experts in the field felt that the complete determination of all finite simple groups—the Classification, as it was termed—was well within reach, and the 18 families plus five sporadic Mathieu groups would constitute the complete list. A blow to this presumption fell around 1965 when Janko discovered a sixth sporadic simple group. A cascade of other sporadic simple groups emerged in subsequent years, sometimes singly and sometimes in mini-families of two or three. The Classification (*9*) was essentially completed around 1980, although a final 1200-page component appeared in print only in 2005.

The Classification theorem states that every finite simple group belongs to one of the following families:

1. The groups of prime order,
2. The alternating groups A_n for $n > 4$,
3. The 16 families of groups of Lie type over finite fields (each has infinitely many members), or
4. The 26 sporadic simple groups.

This culmination—deemed by Gorenstein (*10*) the Enormous Theorem—required 10,000 to 15,000 journal pages spread over some 500 articles, written mostly between 1950 and 1980 by more than 100 mathematicians. This is the genome project of finite group theory! It astounds not only by virtue of its technical complexity, length, and intricacy, but by the emergence of a wholly unexpected and enigmatic gaggle of sporadic exceptions. The rudimentary "elementary particle" structural axioms for group theory have produced an ultimate "atomic" array of simple groups of astonishing beauty, symmetry, and diversity.

The Classification provides an essential foundation for unraveling the structure of finite groups. However, just as molecules and compounds are not completely determined by their list of constituent elements, a given finite group is not uniquely determined only by its list of simple factors; how the factors are "bonded" is crucial. For example, in Fig. 1 the group G of real numbers factors as $G/H = U$, the circle group, where H is the group of integer multiples of 2π; however, this factorization is not "symmetric": There is no subgroup of K of G where G/K is H; moreover, there are many different groups having the same two factors H and U. Constructing larger groups from smaller constituents falls under the rubric of the "extension problem," which is ubiquitous throughout the study of mathematical structures. Even for finite groups, a categorical solution to the extension problem is unattainable, and the universe of finite groups

may be viewed as an infinitely variegated category whose "states" or "molecules" are the groups themselves. In this context, the Classification is a prototype for the study of other complex systems, inasmuch as it provides both structural "germs" and manageable subsystems from which meaningful results may be extracted.

Comparison of the Classification to genome research is not facetious, as the intent, scope, and ramifications of these two massive endeavors are not dissimilar. It would divert us too far afield to list new results that have accrued from the Classification, some within group theory itself but many in other areas of mathematics (*5*). However, one promontory epitomizing the next "layer" of mathematics may be viewed as an outgrowth of the Classification.

The Monster and Moonshine

The largest of the 26 sporadic simple groups is the Fischer-Griess "Monster," containing about 10^{54} elements; it is the nexus of a new level of complexity. Around 1978, McKay noticed striking coincidences between the dimensions of the smallest linear spaces on which the Monster could act and Fourier coefficients of the classical modular function $j(\tau)$, well known from complex analysis and Riemann surface theory (*5*). The smallest nontrivial action (i.e., linear representation) of the Monster occurs in dimension 196,883, which is 1 less than the coefficient of the linear term in the q-series expansion of $j(\tau)$, where $q = \exp(i\tau)$. McKay's observations—which were expanded, honed, and systematized by Thompson, Conway, Norton, and others (*11*)—provided an almost exact one-to-one correspondence between classes of elements in the Monster and certain modular functions associated to Riemann surfaces of genus zero (i.e., compact one-dimensional complex manifolds with no holes). The connections were astonishing and mysterious: remarkable "coincidences" between a structure that emerged from finite group-theoretic research and modular functions over the complex numbers, an area familiar to mathematicians and physicists for more than 100 years. Conway coined the fanciful moniker "Monstrous Moonshine" for them, partly to reflect the slightly "illicit" (and tantalizing) nature of what, at the time, was based almost entirely on dimly lit speculation.

Much work has been done in an attempt to explain the Moonshine connections (*12*), culminating in a full verification of the Moonshine conjectures by Borcherds (*13*), for which he was awarded the Fields Medal in 1998. Building on work of others, Borcherds developed new mathematical structures, now known as Borcherds-Kac-Moody algebras, generalizing the familiar notions of simple Lie algebras, and used these as the cornerstone of his proof. Perhaps even more startling, indeed at yet another "level" of complexity, is Borcherds's use of ideas from perturbative string theory (or conformal field theory) in this work. In hindsight, Gagnnon [(*12*), p. 27] observed, "Almost every facet of Moonshine finds a natural formulation in conformal field theory, where it often was discovered first." Yet although Borcherds established the rigorous mathematics verifying the conjectures, it is perhaps safer to characterize his contribution as shedding more light on, rather than fully illuminating, the root causes for these connections.

There is some speculation that there is a 26-dimensional model of space-time for which the Monster is its group of symmetries. If true, this would be an ultimate layer of complexity on which simple groups have left their imprint. A recent conference (*14*) highlighted the groundbreaking importance of this research: "The [Borcherds-Kac-Moody] algebra $V^{\natural}$ seems to be the natural object to define the Monster and it is conjectured that other (maybe even all) sporadic simple groups arise as automorphism groups of some vertex operator algebras as well. On the other hand, vertex operator algebras are essentially the chiral algebras of conformal quantum field theories and the latter—providing a concept to describe symmetry in two-dimensional critical systems—are one of the objects of greatest interest in modern physics. There exists a special class of quantum field theories called minimal models which are believed to constitute in a certain sense the building blocks of all other conformal field theories and the classification of all such minimal models is actually one of the most important problems in theoretical physics and has attracted growing research interest during the last years." Indeed, in the spirit of Wolfram (*15*), one might speculate even further that some of the fundamental laws of nature or cosmology are, in some fashion yet to be uncovered, compatible with the group axioms, which may then shed some new light on the "unreasonable effectiveness of mathematics."

Conclusion

Scientific research, as with physical systems, evolves over time through generally incremental changes punctuated by breakthroughs, culminations, and unforeseen outcomes. As the field of finite group theory epitomizes, new results in a given area often initiate surges of effort and focus the expenditure of resources; as such, the pursuit of knowledge has facets in common with biological colonies, financial markets, etc. The human element is an essential agent in the evolution of mathematics as a complex system, and the "layers" of complexity mirror the "knowledge states" in this adaptive process. Computers are now taking an increasingly important (and controversial) role in both verifying and discovering new mathematics [see, e.g., (*5*, *16*)]; and so we stand on the threshold of a new dynamic where mathematics, the very foundation of science, may produce and build on results that are humanly unverifiable by even the combined effort of the community, and the veracity of certain results may only be known with some degree of probability.

To reiterate, this case study is intended not just as a primer of advances in finite group theory, but as a test case from which we may glean some general principles for both the characterization and study of complex systems. Evidently, complex systems may evolve from structures according to very elementary rules or transition laws; the seemingly "deterministic" nature of such foundations may belie their ultimate intricacy and unpredictability. A combination of technical depth and breadth of relevance should be essential facets of any complex system. Moreover, each should have "layers" of depth that are reasonably discernible to experts, even if there may be some disagreement about the precise nature of this term or where the "boundaries" of the layers lie. There should be some cross-fertilization of ideas, outcomes, and motivations spanning the layers (even if practitioners work primarily in only one layer).

A complex system must have a substantial impact on systems other than itself; from its study, some larger principles, insights, techniques, or connections should accrue. Emergent behavior is not sufficient to characterize a complex system; rather, a legitimate hallmark is unexpected behavior that leads to deeper understanding of the system or relationships to other phenomena not heretofore considered relevant to the system.

Finally, the study of complex systems should be the exclusive purview of no one but the responsibility of everyone: Each scientist, mathematician, or researcher unfurls the mysteries of nature and humankind in small, deliberate steps. Science embodies the ability to verify, reproduce, and convince others of the veracity of one's discoveries, so the work of scientists is inherently incremental and precise. On the other hand, it is incumbent on us all to work toward enhancing the understanding of "big picture" issues within our own disciplines and beyond; each of our disciplines must itself exhibit the inherent facets of a complex system, or our research is surely nugatory.

References and Notes

1. E. Wigner, *Comm. Pure Appl. Math.* **13**, 1 (1960).
2. P. W. Anderson, *Science* **177**, 393 (1972).
3. G. M. Whitesides, R. F. Ismagilov, *Science* **284**, 89 (1999).
4. G. Weng, U. S. Bhalla, R. Iyengar, *Science* **284**, 92 (1999).
5. See supporting material on *Science* Online.
6. D. Dummit, R. Foote, *Abstract Algebra* (Wiley, Hoboken, NJ, ed. 3, 2004).
7. W. Feit, J. G. Thompson, *Pac. J. Math.* **13**, 775 (1963).
8. J. G. Thompson, *Bull. Am. Math. Soc.* **74**, 383 (1968).
9. D. Gorenstein, R. Lyons, R. Solomon, *AMS Math. Surv. Monogr.* **40**, 1 (1994).
10. D. Gorenstein, *Sci. Am.* **253**, 104 (December 1985).
11. J. Conway, S. Norton, *Bull. London Math. Soc.* **11**, 308 (1979).
12. T. Gagnnon, *Bull. London Math. Soc.* **38**, 1 (2006).
13. R. Borcherds, *Invent. Math.* **109**, 405 (1992).
14. A. A. Ivanov, program for Conference on Algebraic Combinatorics, Monster and Vertex Operator Algebras, Santa Cruz, CA, 24 to 28 October 2000 (www.ma.ic.ac.uk/~ivanov/prog.htm).
15. S. Wolfram, *A New Kind of Science* (Wolfram Media, Champaign, IL, 2002).
16. M. Aschbacher, *Philos. Trans. R. Soc. London Ser. A* **363**, 2401 (2005).

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/410/DC1
SOM Text
References

10.1126/science.1141754

Mitochondrial DNA as a Genomic Jigsaw Puzzle

William Marande and Gertraud Burger*

Mitochondrial genomes exhibit considerable diversity in chromosome form and number as well as coding content (*1*), and they have adopted various modes of transcript maturation, such as RNA editing (*2*) and trans-splicing (*3*). The mitochondrial DNA (mtDNA) of *Diplonema papillatum*, a member of the free-living diplonemids, the sister group of kinetoplastids (*4*), is composed of >100 regularly structured chromosomes 6 kbp (class A) or 7 kbp (class B) in size (*5*). We show that the constant region of each chromosome, which is identical within each size class, covers ~95% of the molecules; a short (<500 bp) variable region (cassette) encloses a distinct gene piece (module) of 60 to 351 bp (Fig. 1A and table S1).

Analysis of genomic (250 kbp) and transcriptome (35 kbp) sequences (*6*) reveals a conventional complement of mitochondrial genes coding for ribosomal RNAs, apocytochrome b, and subunits of cytochrome oxidase, NADH (reduced form of nicotinamide adenine dinucleotide) dehydrogenase, and ATP (adenosine triphosphate) synthase. Without exception, corresponding transcripts are contiguous, whereas the genomic coding regions are fragmented into at least three pieces. For example, the *cox1* gene (specifying cytochrome oxidase subunit 1) consists of nine modules, two of which (modules 1 and 4) are encoded on class B and all others on class A chromosomes (table S1).

How are these systematically modularized genes expressed? Polymerase chain reaction (PCR) amplification with total genomic DNA does not reveal concatenated gene modules, which rules out splicing at the DNA level. Likewise, fragmentation of gene products does not occur, because *Diplonema*'s cDNAs are contiguous. What remains is module concatenation at the RNA level, as experimentally confirmed by Northern hybridization and cDNA sequencing (*6*). In Northern hybridizations of polyadenylated [poly(A)] RNA, a probe containing the C-terminal–most gene module of *cox1* (m9) identifies nine distinct bands: the mature *cox1* mRNA, an RNA species of ~250 nucleotides (nt) (m9 alone), and combined modules 8 plus 9 (m8...m9), 7 plus 8 plus 9 (m7...m9), etc. to m2...m9 (Fig. 1B, lane 1). Similar results are obtained with other *cox1* modules as probes, except that individual transcripts of internal modules show up exclusively in total, not in poly(A), RNA (Fig. 1B, lanes 2 to 5, asterisks). The cDNA library constructed from poly(A) RNA contains the same intermediates seen in Northern experiments (fig. S1). To summarize, (i) gene modules are transcribed individually, (ii) only transcripts of C-terminal gene modules undergo polyadenylation, and (iii) contiguous mRNAs are generated via concatenation of separate module transcripts.

Transcript processing in *Diplonema* mitochondria is different from known trans-splicing (*3*) because sequences adjacent to coding regions are neither conserved nor do they display landmarks of group I, II, or spliceosomal introns. In addition, module junctions do not occur at known organellar intron insertion points, which are otherwise found in highly conserved gene regions. This suggests an RNA processing mechanism, whose detailed biochemistry remains to be uncovered, for *Diplonema*'s discontinuous mitochondrion-encoded genes.

Lastly, we detected potential evidence for RNA editing (Fig. 1C). A run of six nonencoded uridines in the *cox1* transcript between modules 4 and 5 specify amino acids in the deduced Cox1 protein that align unambiguously with homologs in other species. Three lines of evidence support that this insert results from RNA editing rather than a minimodule: (i) gene modules observed in *Diplonema* mitochondria are >10 times longer, (ii) there is no occurrence of six thymidine or adenines in the available ~250-kbp mtDNA sequence, and (iii) uridine-based (insertion and deletion) RNA editing operates in the sister group of diplonemids, the kinetoplastids (*7*). We posit that RNA editing in *Diplonema* is directed by (yet undetected) guide RNAs but that their primary role is alignment of cognate module transcripts. Thus, we hypothesize that transcript maturation in *Diplonema* mitochondria requires ligase, nuclease, and helicase—activities present in kinetoplastid editosomes (*7*).

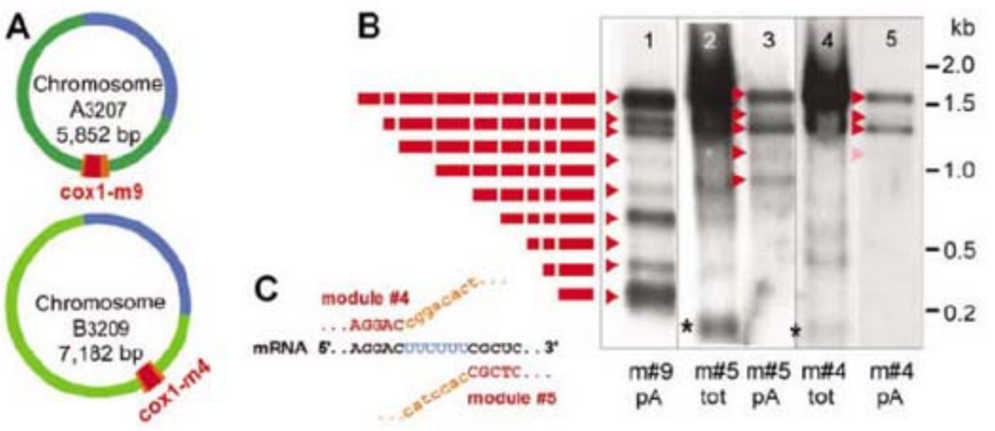


Fig. 1. (**A**) Representative mitochondrial chromosomes of *Diplonema*. Green and blue arcs are the constant region; the green portion is identical in chromosomes of the same class; the blue portion is identical between chromosomes of class A and B. Cassettes include a gene module (red box) and flanking regions (orange boxes). (**B**) Northern hybridization with polyadenylated (pA) or total RNA (tot). Probes are *cox1* coding regions, i.e., the C-terminal (m9, lane 1) and two internal modules (m5, lanes 2 and 3; m4, lanes 4 and 5). Triangles point to bands, and asterisks indicate transcripts of individual modules. The presence of bands in lanes 2 and 4 that are not seen in lane 1 indicates ligation of non-poly(A) transcripts and suggests that different pathways of module assembly exist. Rows of red boxes symbolize polyadenylated transcript intermediates. (**C**) *cox1* mRNA with nonencoded uridines (blue) between m4 and m5. Genomic sequences of gene modules and module-flanking regions are indicated in red uppercase and orange lowercase letters, respectively.

References and Notes

1. M. W. Gray, B. F. Lang, G. Burger, *Annu. Rev. Genet.* **38**, 477 (2004).
2. M. W. Gray, *IUBMB Life* **55**, 227 (2003).
3. L. Bonen, *FASEB J.* **7**, 40 (1993).
4. A. G. B. Simpson, A. J. Roger, *Mol. Phylogenet. Evol.* **30**, 201 (2004).
5. W. Marande, J. Lukes, G. Burger, *Eukaryot. Cell* **4**, 1137 (2005).
6. Materials and methods are available on *Science* Online.
7. K. D. Stuart, A. Schnaufer, N. L. Ernst, A. K. Panigrahi, *Trends Biochem. Sci.* **30**, 97 (2005).

8. This work was supported by the Canadian Institutes of Health Research Institute of Genetics (MOP-79309) and the Canadian Institute for Advanced Research. GenBank accession nos. are AY686226 and EU123536 to EU123538.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/415/DC1
Materials and Methods
SOM Text
Fig. S1
Table S1
References

19 July 2007; accepted 29 August 2007
10.1126/science.1148033

Department of Biochemistry, Université de Montréal, Montréal, Quebec H3T 1J4, Canada.

*To whom correspondence should be addressed. E-mail: Gertraud.Burger@UMontreal.ca

Mass-Dependent and -Independent Fractionation of Hg Isotopes by Photoreduction in Aquatic Systems

Bridget A. Bergquist and Joel D. Blum

Mercury (Hg) isotopes can be used as tracers of Hg biogeochemical pathways in the environment. The photochemical reduction of aqueous Hg species by natural sunlight leads to both mass-dependent fractionation (MDF) of Hg isotopes and mass-independent fractionation (MIF) of the odd-mass isotopes, with the relation between the MIF for the two odd isotopes being distinct for different photoreduction pathways. Large variations in MDF and MIF are observed in fish and provide new insights into the sources and bioaccumulation of Hg in food webs. MIF in fish can also be used to estimate the loss of methylmercury via photoreduction in aquatic ecosytems.

Mercury (Hg) is a globally distributed and extremely toxic pollutant (*1*, *2*), and its mobility and bioaccumulation are highly dependent on speciation and reductive-oxidative cycling in the environment (*3*). Both inorganic and methylated Hg species have severe health effects in humans, but it is monomethylmercury (MeHg) that is thought to be the main species involved in bioaccumulation in the food web and human exposure via fish consumption (*4*). Human activities, such as coal combustion, and natural Hg fluxes release large amounts of inorganic Hg to the atmosphere either as a gas (Hg^0) or as the reactive Hg^{+2} species (*2*, *3*). In aquatic systems and sediments, a small fraction of the Hg^{+2} is transformed to the bioavailable MeHg form (*5*). Although Hg poses a proven health risk, much of the natural cycle of Hg is not well understood (*3*), and new approaches are necessary to better elucidate, track, and quantify Hg sources and transformations in the environment.

Stable isotope fractionation of light elements (C, H, O, S, and N) is widely used to identify and trace biogeochemical pathways. Recent analytical advancements, especially in multicollector inductively coupled plasma mass spectrometry (MC-ICPMS), have allowed for stable isotopic studies of many heavier elements [>40 atomic mass units (amu)], including Hg. Hg has seven stable isotopes (196, 198, 199, 200, 201, 202, and 204 amu) with a mass difference of 4%, active redox chemistry, a volatile form (Hg^0), and a tendency to form covalent bonds, thus providing many opportunities for isotopic fractionation. A large range in $\delta^{202}Hg$ values is observed in natural samples, with most samples displaying mass-dependent fractionation (MDF) (*6*–*12*). A small body of data also suggests that natural samples sometimes display mass-independent fractionation (MIF) of Hg isotopes (*13*, *14*). Only a few other isotopic systems have been shown to display MIF, the most notable of which are O and S (*15*–*17*). In both cases, the application of MIF has proven very useful in a number of fields, including cosmochemistry, paleoclimatology, physical chemistry, atmospheric chemistry, and biogeochemistry.

We documented MIF of Hg isotopes during a natural process, constrained the potential mechanism of isotope fractionation, and applied the MIF observed in natural samples to quantify the photochemical reduction of Hg species in the environment. The reduction of Hg species to Hg^0 vapor is an important pathway for the transfer of Hg from aquatic systems to the atmosphere (*18*, *19*) and can occur by photochemical (*20*–*22*), biological (*23*, *24*), or abiotic organically mediated (*25*) mechanisms. Understanding the controls and determining the relative importance of the different reduction mechanisms is important to quantifying how much Hg is retained within an ecosystem that can be methylated and/or bioaccumulated.

Photochemical reaction experiments and MIF. We studied photochemical reduction of aqueous Hg^{+2} and MeHg by natural sunlight in a quartz reaction vessel with dissolved organic carbon (DOC). Photoreduction resulted in both MDF and MIF of Hg isotopes. In all the experiments, isotopic fractionation followed Rayleigh-type fractionation in such a way that lighter isotopes were preferentially reduced and removed from the vessel. Although the rate of the MeHg photoreduction was slower (a 20% loss after 300 min) than the rate of Hg^{+2} photoreduction (>90% loss after 300 min), the MeHg experiments resulted in larger isotopic fractionation ($^{202/198}\alpha_{product/reactant}$ = 0.9987 and 0.9983) than the Hg^{+2} experiments (α = 0.9994). No detectable MeHg was reduced during the dark abiotic control experiment, but 20% of the Hg^{+2} was reduced after 300 min in the Hg^{+2} dark control experiments, and the resulting isotopic fractionation was larger than in Hg^{+2} photoreduction (α = 0.9980 and 0.9987). This dark abiotic Hg^{+2} reduction probably resulted from organically mediated reactions (*25*). The reduction experiments were performed at Hg concentrations above environmental levels (60 to 100 ng/g) and with continuous sparging and removal of the produced Hg^0 from the reaction vessel, which hindered Hg^0 photo-oxidation. Therefore, reduction

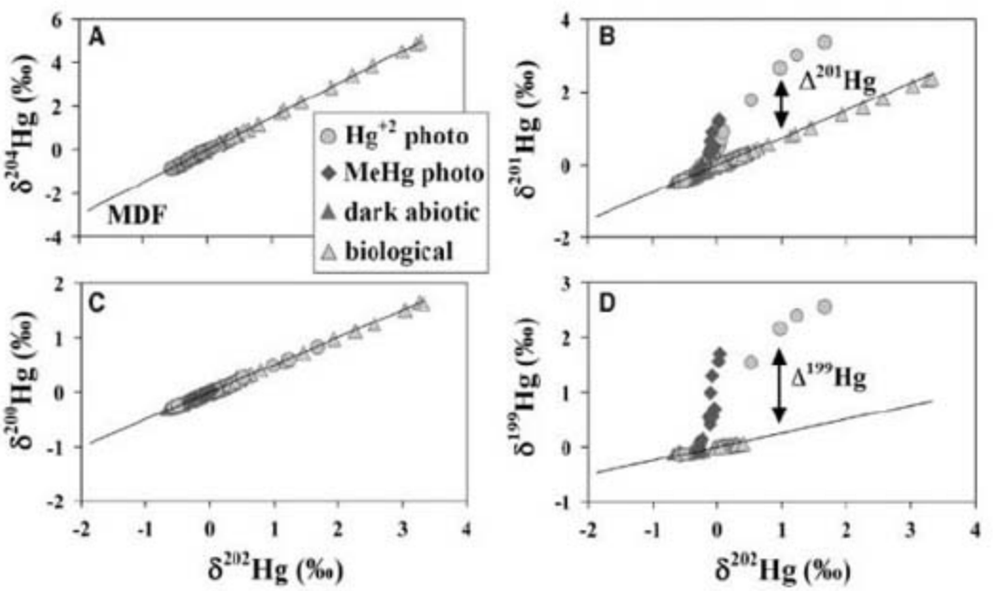


Fig. 1. Hg isotopic compositions, with $\delta^{202}Hg$ plotted against (**A**) $\delta^{204}Hg$, (**B**) $\delta^{201}Hg$, (**C**) $\delta^{200}Hg$, and (**D**) $\delta^{199}Hg$. Shown are of the Hg^{+2} photoreduction experiments, the MeHg photoreduction experiments, and dark controls from this study along with biological reduction experiments (*12*). The solid line plotted in each is the theoretically predicted MDF based on the $\delta^{202}Hg$ value (*40*, *41*). Isotopic compositions and MDF are reported in delta notation relative to a National Institute of Standards and Technology Hg standard (SRM 3133) with ^{198}Hg in the denominator: $\delta^{xxx}Hg = 1000 \times [(^{xxx/198}R_{sample}/^{xxx/198}R_{SRM\ 3133}) - 1]$, where xxx is the mass of each mercury isotope between 199 and 204 amu. For natural samples, the reproducibility of replicate analyses is typically better than ±0.15‰ (2σ, SE) for $\delta^{202}Hg$.

Department of Geological Sciences, University of Michigan, 1100 North University Avenue, Ann Arbor, MI 48109, USA.

rates measured in our experiments are higher than rates observed in nature, and the resulting fractionation factors may not be directly applicable to natural systems. Details of the methods, nomenclature, and fractionation factors modeled for all of the reduction experiments are summarized in the supporting online material (SOM) (*26*).

The most striking observation of our experiments is that only photochemical reduction resulted in dramatic MIF [up to 2.5 per mil (‰)], and it did so only for the odd isotopes of Hg (Fig. 1). In contrast, the dark abiotic reduction experiments (this study), and the biological reduction experiments from Kritee *et al.* (*12*) followed only MDF for both the even and odd isotopes. As the photoreduction of Hg progressed, odd isotopes were preferentially retained in the reactor, with the slower MeHg photoreduction experiments displaying more MIF than the Hg^{+2} photoreduction at the same extent of reaction (Fig. 2). Furthermore, although the overall rate of reduction of MeHg in each photoreduction experiment did not change significantly with different concentrations of DOC (1 and 10 mg of C/liter; see SOM), the degree of MIF was larger for the higher-DOC experiment.

The relation between the MIF for the two odd isotopes can be used to distinguish the different photoreduction pathways and could allow for the identification of specific chemical pathways in nature. Plotting Δ^{199}Hg versus Δ^{201}Hg reveals a significant difference in the relation between the two odd isotopes for the photoreduction of Hg^{+2} versus MeHg (Fig. 3). For the Hg^{+2} photoreduction, Δ^{199}Hg and Δ^{201}Hg have similar values at the same extent of reaction. However, Δ^{199}Hg is larger than Δ^{201}Hg for the MeHg photoreduction experiments. Despite the fact that the two MeHg photoreduction experiments (1 and 10 mg of C/liter) display different degrees of MIF at the same extent of reaction (Fig. 2), the relation between Δ^{199}Hg and Δ^{201}Hg is the same for both experiments (Fig. 3). This result suggests that the reaction step that resulted in MIF for the MeHg experiments is dependent on the amount of DOC, but it is probably the same mechanism causing MIF in both MeHg experiments.

The observation of large MIF in the photochemical reduction experiments raises the question of the mechanism of isotope fractionation. MDF arises from differences in zero-point vibrational energies and thus differences in bond strengths of isotopes due to their differing masses, and can be expressed in both kinetic and equilibrium processes (*27*, *28*). Isotopic fractionation that does not follow MDF is both predicted and observed (*15*–*17*, *29*–*33*). Two of the plausible mechanisms relevant to Hg MIF are the nuclear field shift effect (*29*, *33*) and the magnetic isotope effect (*30*–*32*).

Both the nuclear field shift effect and the magnetic isotope effect predict differences in isotope fractionation between even and odd isotopes that are mass-independent. Deviation from mass-dependent scaling between isotopes occurs in the nuclear field shift effect because this effect is dependent on the nuclear volume and nuclear charge radius, which do not scale linearly with the number of neutrons. Even/odd staggering of the isotopes results because the odd isotopes have ground-state energies closer to those of the adjacent lower-mass even isotope. Nuclear field effects become more important with the increasing mass of an element and should be significant for heavier elements such as U, Hg, and Tl (*29*, *33*). In a recent modeling study (*33*), it was predicted that equilibrium isotope fractionation of Hg due to the nuclear field shift effect should be larger than mass-dependent effects, and the MIF attributable to the nuclear field shift effect should be largest for ^{199}Hg and ^{204}Hg and to a lesser extent for ^{201}Hg and ^{196}Hg.

The magnetic isotope effect is predicted to result in a more pronounced difference in the behavior of odd versus even isotopes. Only the odd isotopes have nonzero nuclear spin, nuclear magnetic moments, and hyperfine splitting. This makes the reaction rates of radical intermediates that undergo spin conversion significantly different for radical pairs that involve magnetic nuclei versus nonmagnetic nuclei (*30*–*32*). Thus, the magnetic and nonmagnetic isotopes can be preferentially accumulated in different reaction products in chemical processes with radical intermediates

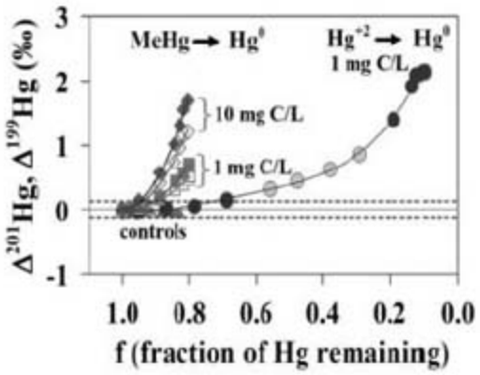


Fig. 2. MIF of reactor samples from the photoreduction experiments plotted as a function of the fraction of Hg remaining in the reactor. Three experiments along with dark abiotic controls are plotted: (●) photoreduction of Hg^{+2} with 1 mg of C/liter of DOC, (■) photoreduction of MeHg with 1 mg of C/liter of DOC, (◆) photoreduction of MeHg with 10 mg of C/liter of DOC, and (▲) dark abiotic controls. Δ^{199}Hg is plotted with the solid symbols and Δ^{201}Hg with lighter shaded symbols. MIF is reported using capital delta notation and is the deviation in the measured isotope ratios from the theoretical value based on mass-dependent kinetic isotope fractionation. δ^{202}Hg is used to establish the theoretical mass-dependent values for δ^{199}Hg and δ^{201}Hg according to the kinetic mass-dependent fractionation law (*40*, *41*), and the theoretical MDF is subtracted from the measured isotopic composition using the following formulas: $\Delta^{199}\text{Hg} = 1000 \times [(\ln((\delta^{199}\text{Hg}/1000) + 1)) - 0.2520 \times (\ln((\delta^{202}\text{Hg}/1000) + 1))]$; $\Delta^{201}\text{Hg} = 1000 \times [(\ln((\delta^{201}\text{Hg}/1000) + 1)) - 0.7520 \times (\ln((\delta^{202}\text{Hg}/1000) + 1))]$. The reproducibility of replicate analyses for natural samples for Δ^{199}Hg and Δ^{201}Hg is typically better than ±0.10‰ (2σ, SE).

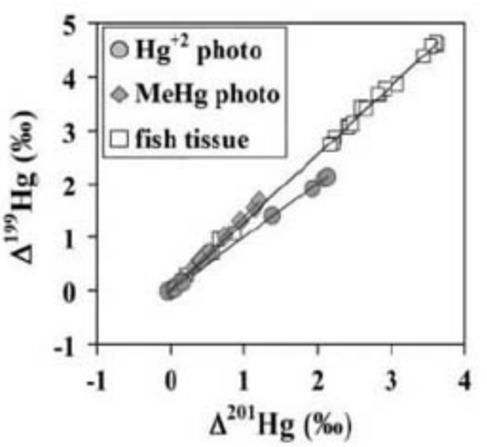


Fig. 3. Δ^{201}Hg versus Δ^{199}Hg for the photochemical reduction experiments and fish tissue samples. Dark abiotic controls all plot at the origin. The fish tissue samples plotted are from three sites in Lake Michigan. Marine dogfish (*Squalus acanthias*) reference standards [DORM-2 (muscle) and DOLT-2 (liver), see SOM] are also shown. The New England lake fish are not included because only Δ^{201}Hg was measured for these samples. The slopes are 1.36 (±0.02, 2 SE) for the MeHg photoreduction experiments, 1.00 (±0.02, 2 SE), for the Hg^{+2} photoreduction experiment, and 1.28 (±0.03, 2 SE) for the fish tissue samples.

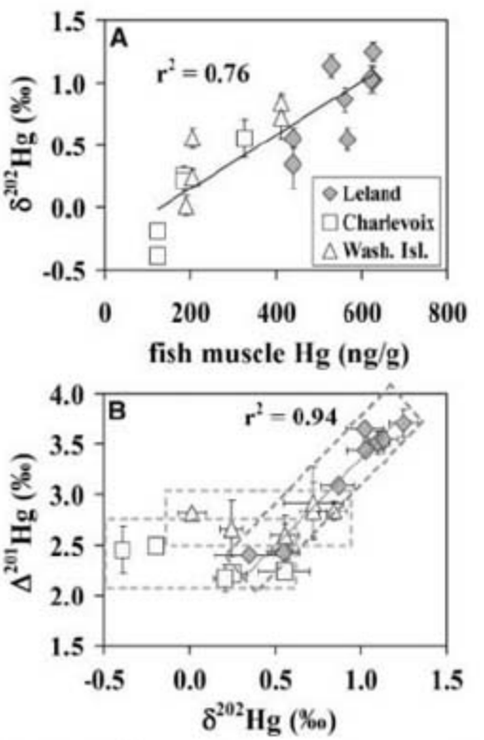


Fig. 4. (**A**) δ^{202}Hg plotted as a function of the Hg concentration (nanograms/gram) in fish muscle for burbot fish (*Lota lota*) sampled from Lake Michigan. (**B**) The MIF (Δ^{201}Hg) plotted against the MDF (δ^{202}Hg) for the same fish samples as in (A). Fish samples were collected at three sites in Lake Michigan: Leland, Charlevoix, and Washington Island. Also shown are the correlation coefficients (r^2) for all the fish samples in (A) and for the Leland site in (B).

that are branching and/or reversible. For example, in a reaction where a radical pair starts in a triplet state, the radical pairs with magnetic nuclei undergo more rapid triplet-to-singlet state conversion than the radical pairs with nonmagnetic nuclei and may preferentially recombine to regenerate the starting molecule (*31*, *32*). In contrast to the nuclear field shift effect, which can be expressed in both equilibrium and kinetic reactions, the magnetic isotope effect is purely a kinetic phenomenon.

Although we cannot unequivocally differentiate between the nuclear field shift effect and magnetic isotope effect, or a combination of these effects, it appears most likely that the magnetic isotope effect is responsible for the MIF observed in the photochemical reactions conducted in this study. The proposed mechanisms for photochemical reactions of Hg and DOC include radical intermediates such as Hg^{+} and $^{\bullet}HgCl$ (*22*, *34*), which would facilitate the magnetic isotope effect. Another observation in support of the magnetic isotope effect as the likely MIF mechanism in the photoreduction experiments is the difference in the relations between $\Delta^{199}Hg$ and $\Delta^{201}Hg$ for different reaction pathways (Fig. 3). The nuclear field shift does not provide a clear mechanism for differing fractionation patterns for different reaction pathways. The nuclear field shift effect also predicts MIF anomalies in $\Delta^{204}Hg$ (*33*), which we did not observe.

MDF and MIF of Hg isotopes in the environment. The observation of MDF and MIF in laboratory experiments raises the possibility of applying Hg isotopic variations to understand Hg in environmental systems. For instance, having an isotopic tool to help understand Hg bioaccumulation would be useful in the same way that $\delta^{15}N$ is used in food web studies (*35*). $\delta^{202}Hg$, a measure of MDF, is strongly correlated with fish muscle Hg concentrations for burbot fish (*Lota lota*) from Lake Michigan (Fig. 4A). Because the Hg concentration in fish muscle generally correlates with the size, age, and trophic level of a fish (*36*, *37*), this result suggests that $\delta^{202}Hg$ is also related to these parameters. Additionally, the Hg in fish becomes isotopically heavier with increasing Hg concentration, indicating that isotopically light Hg is excreted by fish as they age. Thus, MDF of Hg traces Hg bioaccumulation and excretion in fish.

MDF, however, is not the only isotopic signature preserved in fish. We have also found that fish display a large range in MIF (up to 4‰ in $\Delta^{201}Hg$). In a plot of $\delta^{202}Hg$ against $\Delta^{201}Hg$ (Fig. 5), representing MDF against MIF, three groups of fish samples (from Lake Michigan, New England lakes, and the ocean) occupy different regions of the plot. Different Hg sources and/or Hg transformations in aquatic ecosystems may explain contrasts in the MIF and MDF between these groups of fish. Despite these differences in the extent of MIF and MDF found in the fish muscle samples, the relation between the MIF of the two odd isotopes in all of the fish has a similar slope to that found for photoreduction of MeHg (Fig. 3). This is consistent with the finding that fish bioaccumulate mostly MeHg and that a majority (>80%) of Hg present in fish muscle is MeHg (*38*).

The similar slopes of $\Delta^{199}Hg$ versus $\Delta^{201}Hg$ for the MeHg photoreduction experiments and the fish samples suggest that the MIF observed in fish is a signature imparted to the MeHg while it was in the photic zone before it was incorporated into the food web, and that the original MIF of the MeHg remained unperturbed despite additional MDF processes occurring within the food web. This interpretation is also supported by results from two sites in Lake Michigan, for which all of the fish from a given locale have similar MIF despite large ranges in MDF (Fig. 4B). At a third site in Lake Michigan, there was a strong correlation between $\delta^{202}Hg$ and $\Delta^{201}Hg$ (Fig. 4B). Although this could indicate that there are processes during bioaccumulation in the food web that produce both MDF and MIF, it is more likely that the correlation represents a mixing line between two different sources of MeHg with two distinct signatures of MIF for this group of fish. These correlations of MDF with the size, age, and trophic level of fish, along with the MIF signatures in fish, can potentially be used to understand the sources and cycling of Hg in addition to bioaccumulation of Hg in food webs.

MIF in fish could further be used to quantify the loss of MeHg via photoreduction in aquatic ecosystems. Using the MeHg photoreduction experiment (1 mg of C/liter) and the relation between the fraction of Hg remaining in solution and $\Delta^{201}Hg$, a Rayleigh distillation model can be fit to the data and a relation derived between these parameters. This relation can then be applied to the $\Delta^{201}Hg$ preserved in fish tissues to estimate the MeHg lost via photoreduction in the ecosystem. The MIF in Lake Michigan fish suggests that ~68 ± 8% (1 SD) of the MeHg was lost via photochemical reduction before incorporation into the food web. The New England lake fish are of several species from two small lakes, and estimated losses are smaller, ~25 ± 8% and ~55 ± 8% of MeHg, for the two lakes. In general, the magnitude of the above MeHg losses via photoreduction agrees with other estimates from freshwater systems (*21*, *39*) and supports the hypothesis that this is an important pathway for the removal of MeHg from aquatic ecosystems. However, we caution that the relation between MIF and the fraction of MeHg lost via photoreduction depends on the concentration of DOC (and possibly the type of DOC) and may be site-specific, so the estimates given here will need to be confirmed with experiments using natural water from specific sites.

Implications for the global Hg cycle. Because fish, and presumably other organisms in aquatic food webs, accumulate Hg with positive MIF (enriched in the odd isotopes), a complementary reservoir must exist with negative MIF Hg (depleted in the odd isotopes). If positive MIF Hg is retained in the aqueous system after photoreduction of Hg species, then negative MIF Hg may be released to the atmosphere as Hg^0. Because Hg cycles between several active pools near Earth's surface and Hg^0 has an atmospheric residence time of about 1 year (*3*), there should be the opportunity for isotopically distinct Hg^0 to be transported away from the aquatic systems in which it was produced and be deposited elsewhere. The preservation of both MDF and MIF in environmental reservoirs of Hg will depend on the mass balance of Hg sources, but the observation of even a small degree of MIF in soils or other atmospherically derived pools would suggest that photochemical cycling of Hg species plays a substantial role in the global cycle of Hg. Thus, we expect that the MDF and MIF recorded in natural environmental samples will contribute to the understanding and quantification of important processes in the global cycle of Hg.

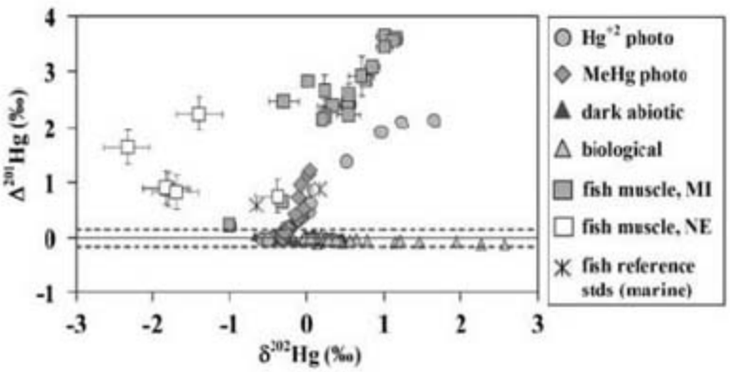


Fig. 5. MIF ($\Delta^{201}Hg$) plotted as a function of MDF ($\delta^{202}Hg$). Hg isotopic data are plotted from the photoreduction experiments, dark abiotic controls, fish muscle samples from Lake Michigan (MI) and New England (NE) lakes, and marine dogfish (*Squalus acanthias*) reference standards [DORM-2 (muscle) and DOLT-2 (liver), see SOM]. The biological reduction experiments (*12*) are plotted as well. The fish tissue samples from Lake Michigan are from burbot fish (*Lota lota*) muscle and from three locations. The fish muscle samples from the New England lakes are from two different lakes and from three species [yellow perch (*Perca flavescens*), chain pickerel (*Esox niger*), and pumpkinseed (*Lepomis gibbosus*)]. Location and sample details can be found in the SOM. Error bars are 2σ errors (2 SE) of replicate analysis except for the New England lake fish samples. These New England lake fish samples were measured earlier in the development of the method without replicates, and larger uncertainties of ±0.3‰ for $\delta^{202}Hg$ and ±0.2‰ for $\Delta^{201}Hg$ were applied to these samples.

References and Notes

1. W. F. Fitzgerald, D. R. Engstrom, R. P. Mason, E. A. Nater, *Environ. Sci. Technol.* **32**, 1 (1998).
2. C. H. Lamborg *et al.*, *Global Biogeochem. Cycles* **16**, 1104 (2002).
3. W. F. Fitzgerald, C. H. Lamborg, *Treatise Geochem.* **9**, 107 (2003).

4. T. W. Clarkson, *Environ. Health Perspect.* **100**, 31 (1993).
5. S. M. Ullrich, T. W. Tanton, S. A. Abdrashitova, *Crit. Rev. Environ. Sci. Technol.* **31**, 241 (2001).
6. D. S. Lauretta, B. Klaue, J. D. Blum, P. R. Buseck, *Geochim. Cosmochim. Acta* **65**, 2807 (2001).
7. H. Hintelmann, S. Y. Lu, *Analyst* **128**, 635 (2003).
8. H. Hintelmann, N. Ogrinc, *Am. Chem. Soc. Symp. Ser. Biogeochem. Environ. Important Trace Elements* **835**, 321 (2003).
9. T. A. Jackson, D. C. G. Muir, W. F. Vincent, *Environ. Sci. Technol.* **38**, 2813 (2004).
10. C. N. Smith, S. E. Kesler, B. Klaue, J. D. Blum, *Geology* **33**, 825 (2005).
11. D. Foucher, H. Hintelmann, *Anal. Bioanal. Chem.* **384**, 1470 (2006).
12. K. Kritee, J. D. Blum, M. W. Johnson, B. A. Bergquist, T. Barkay, *Environ. Sci. Technol.* **41**, 1889 (2007).
13. B. A. Bergquist, J. D. Blum, M. W. Johnson, A. Biswas, *Eos* **52**, Abstr. V13E-06 (2006).
14. T. A. Jackson, *Geochim. Cosmochim. Acta* **70** (suppl.), A284 (2006).
15. M. H. Thiemens, J. E. I. Heidenreich, *Science* **219**, 1073 (1983).
16. J. Farquhar, H. M. Bao, M. H. Thiemens, *Science* **289**, 756 (2000).
17. M. H. Thiemens, *Annu. Rev. Earth Planet. Sci.* **34**, 217 (2006).
18. J. P. Kim, W. F. Fitzgerald, *Science* **231**, 1131 (1986).
19. G. M. Vandal, R. P. Mason, W. F. Fitzgerald, *Water Air Soil Pollut.* **56**, 791 (1991).
20. M. Amyot, G. Mierle, D. R. S. Lean, D. J. McQueen, *Environ. Sci. Technol.* **28**, 2366 (1994).
21. P. Sellers, C. A. Kelly, J. W. M. Rudd, A. R. MacHutchon, *Nature* **380**, 694 (1996).
22. H. Zhang, *Struct. Bond Recent Dev. Mercury Sci.* **120**, 37 (2006).
23. R. P. Mason, F. M. M. Morel, H. F. Hemond, *Water Air Soil Pollut.* **80**, 775 (1995).
24. T. Barkay, J. K. Schaefer, A. J. Poulain, M. Amyot, *Geochim. Cosmochim. Acta* **69**, A702 (2005).
25. B. Allard, I. Arsenie, *Water Air Soil Pollut.* **56**, 457 (1991).
26. Additional details on experimental methods, nomenclature, and calculations are in the SOM on *Science* Online.
27. J. Bigeleisen, M. Mayer, *J. Chem. Phys.* **15**, 261 (1947).
28. H. C. Urey, *J. Chem. Soc.* **47**, 562 (1947).
29. J. Bigeleisen, *J. Am. Chem. Soc.* **118**, 3676 (1996).
30. A. L. Buchachenko, *J. Phys. Chem.* **105**, 9995 (2001).
31. V. Berdinskii, L. Yasina, A. Buchachenko, *Russ. J. Phys. Chem.* **78**, 261 (2004).
32. A. L. Buchachenko *et al.*, *Dokl. Phys. Chem.* **413**, 39 (2007).
33. E. A. Schauble, *Geochim. Cosmochim. Acta* **71**, 2170 (2007).
34. M. Ravichandran, *Chemosphere* **55**, 319 (2004).
35. M. Minagawa, E. Wada, *Geochim. Cosmochim. Acta* **48**, 1135 (1984).
36. C. A. Bache, W. H. Gutenman, D. J. Lisk, *Science* **172**, 951 (1971).
37. K. A. Kidd, R. H. Hesslein, R. J. P. Fudge, K. A. Hallard, *Water Air Soil Pollut.* **80**, 1011 (1995).
38. K. Kannan *et al.*, *Arch. Environ. Contam. Toxicol.* **34**, 109 (1998).
39. C. R. Hammerschmidt, W. F. Fitzgerald, *Environ. Sci. Technol.* **40**, 1212 (2006).
40. E. D. Young, A. Galy, H. Nagahara, *Geochim. Cosmochim. Acta* **66**, 1095 (2002).
41. J. D. Blum, B. A. Bergquist, *Anal. Bioanal. Chem.* **388**, 353 (2007).

42. We thank M. Johnson, S. Long, and A. Biswas for support and assistance in experiments and sample processing; D. Jude and C. Chen for providing fish tissue samples; and T. Barkay, K. Kritee, G. Slater, and J. Farquhar for discussions. We also thank the reviewers for their helpful comments. Funding was provided by NSF grant EAR-0433793 to J.D.B., and B.A.B. was partially supported by a Turner Postdoctoral Fellowship from the Department of Geological Sciences at the University of Michigan.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1148050/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 to S4
References

19 July 2007; accepted 5 September 2007
Published online 13 September 2007;
10.1126/science.1148050
Include this information when citing this paper.

Paired-End Mapping Reveals Extensive Structural Variation in the Human Genome

Jan O. Korbel,[1,2]* Alexander Eckehart Urban,[3]* Jason P. Affourtit,[4]* Brian Godwin,[4] Fabian Grubert,[5] Jan Fredrik Simons,[4] Philip M. Kim,[1] Dean Palejev,[5] Nicholas J. Carriero,[6] Lei Du,[4] Bruce E. Taillon,[4] Zhoutao Chen,[4] Andrea Tanzer,[7,8,9] A. C. Eugenia Saunders,[3] Jianxiang Chi,[10] Fengtang Yang,[10] Nigel P. Carter,[10] Matthew E. Hurles,[10] Sherman M. Weissman,[5] Timothy T. Harkins,[11] Mark B. Gerstein,[1,6,12] Michael Egholm,[4]† Michael Snyder[1,3]†

Structural variation of the genome involves kilobase- to megabase-sized deletions, duplications, insertions, inversions, and complex combinations of rearrangements. We introduce high-throughput and massive paired-end mapping (PEM), a large-scale genome-sequencing method to identify structural variants (SVs) ~3 kilobases (kb) or larger that combines the rescue and capture of paired ends of 3-kb fragments, massive 454 sequencing, and a computational approach to map DNA reads onto a reference genome. PEM was used to map SVs in an African and in a putatively European individual and identified shared and divergent SVs relative to the reference genome. Overall, we fine-mapped more than 1000 SVs and documented that the number of SVs among humans is much larger than initially hypothesized; many of the SVs potentially affect gene function. The breakpoint junction sequences of more than 200 SVs were determined with a novel pooling strategy and computational analysis. Our analysis provided insights into the mechanisms of SV formation in humans.

Structural variation of large segments (>50 kb) of the human genome was recently found to be widespread in healthy individuals (*1*–*4*), with ~4000 affected genomic loci currently listed in the Database of Genomic Variants (DGV) (*2*). Structural variants (SVs) may have a more significant impact on phenotypic variation than single-nucleotide polymorphisms (SNPs) (*4*, *5*). SVs have been implicated in gene expression variation (*5*), female fertility (*6*), susceptibility to HIV infection (*7*), systemic autoimmunity (*8*), and genomic disorders such as Williams-Beuren syndrome and velocardiofacial syndrome (*9*, *10*). Thus, understanding the full extent of structural variation is important for understanding phenotypic variation and genetic disease in humans.

Previous methods for detecting SVs used comparative genome hybridization—array-CGH, which involves DNA microarrays and detects copy-number variants, or CNVs (*4*) and fosmid paired-end sequencing (FPES) (*3*)—at relatively low resolution (>50 kb for array-CGH, >8 kb for FPES). Note that these methods map SVs below the resolution where breakpoints can be detected (for array-CGH) or are laborious (for FPES). Consequently, breakpoint junction sequences of a limited number of SVs and/or CNVs have been reported (*2*, *3*, *11*). Methods for comprehensively detecting SVs of <10 kb, which may encompass most variants, and for mapping breakpoints are lacking; thus, how SVs affect genes and the mechanisms by which SVs form are not known.

Development of paired-end mapping for detecting SVs. In order to identify SVs more accurately, we developed paired-end mapping (PEM), which involves the preparation and isolation of paired ends of 3-kb fragments (*12*), and their massive sequencing with 454 technology (Fig. 1) (*13*). The large number of paired-end reads was optimally mapped to the human genome computationally (*12*). Structural rearrangements were identified as significant differences between the fragments identified by the paired-end reads and the corresponding regions of the reference sequence. Five different signatures (i to v) were used to predict SVs (*12*) (Fig. 1B). (i) Deletions relative to the reference genome were identified by paired ends spanning a genomic region in the reference genome longer

[1]Molecular Biophysics and Biochemistry Department, Yale University, New Haven, CT 06520, USA. [2]European Molecular Biology Laboratory, 69117 Heidelberg, Germany. [3]Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT 06520, USA. [4]454 Life Sciences, A Roche Company, Branford, CT 06405, USA. [5]Department of Genetics, Yale University School of Medicine, New Haven, CT 06520, USA. [6]Department of Computer Science, Yale University, New Haven, CT 06520, USA. [7]Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA. [8]Department of Computer Science, University of Leipzig, 04107 Leipzig, Germany. [9]Institute for Theoretical Chemistry, University of Vienna, 1090 Vienna, Austria. [10]The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK. [11]Roche Applied Science, Indianapolis, IN 46250, USA. [12]Program in Computational Biology and Bioinformatics, Yale University, New Haven, CT 06520, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: megholm@454.com (M.E.); michael.snyder@yale.edu (M.S.)

than a specified cutoff (Fig. 1). (ii) Simple insertions relative to the reference genome were predicted with paired ends that spanned a region shorter than a cutoff. (iii) Mated insertions contained sequences connected to a distal locus on the basis of their paired ends. (iv) Inversions were detected through a relative orientation different from the reference genome. (v) Unmated insertions contained sequences connected to a distal

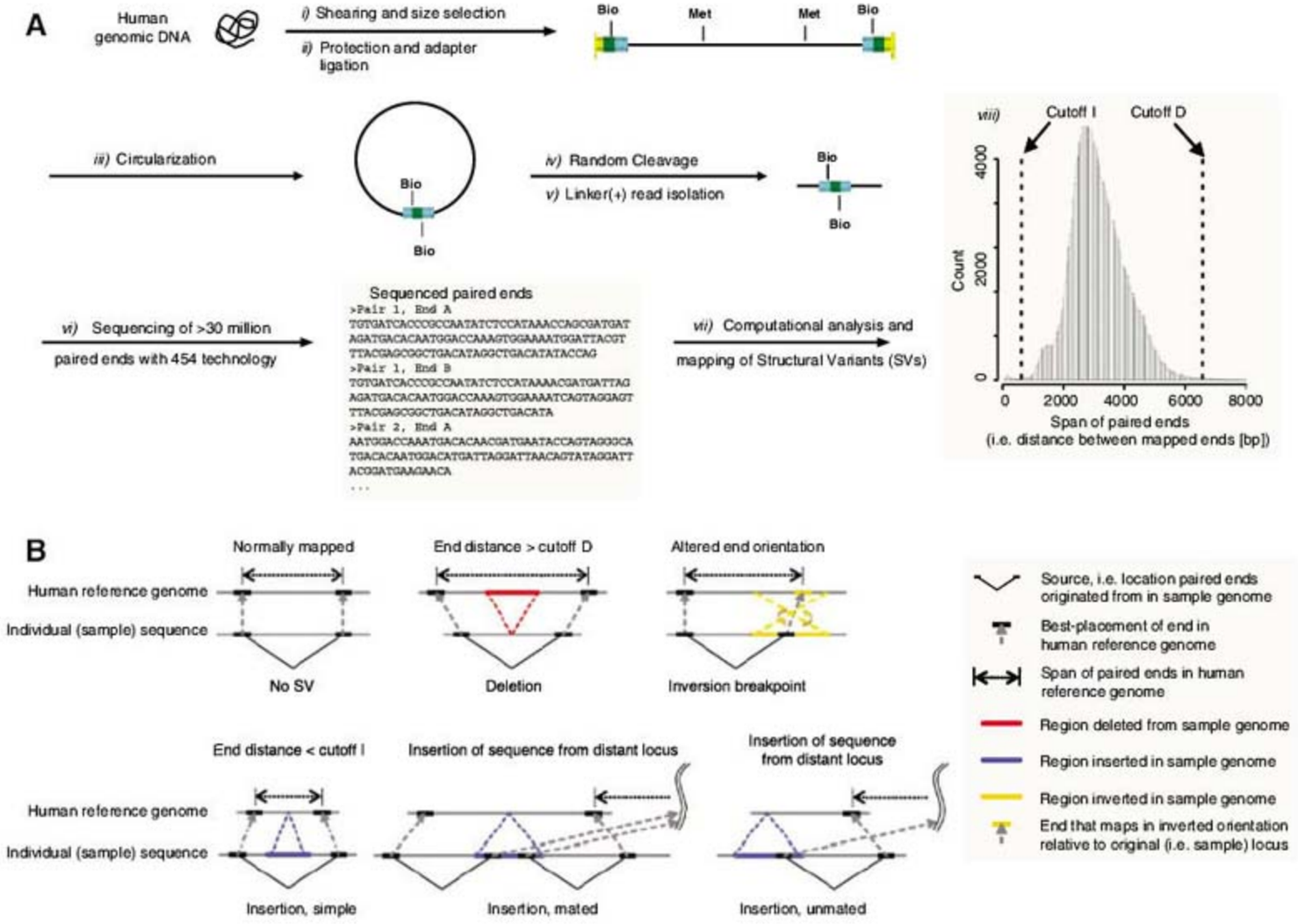


Fig. 1. (**A**) Flow chart illustrating PEM. (i) Genomic DNA was sheared to yield DNA fragments ~3 kb; (ii) biotinylated hairpin adapters were ligated to the fragment ends; (iii) fragments were circularized (iv) and randomly sheared; (v) linker (+) fragments were isolated; (vi) the library was subjected to 454 sequencing (*13*). (vii) Paired ends were analyzed computationally to determine (viii) the distribution of "paired-end spans" (shown for a single 454 sequencing pool). (**B**) Types of SVs. Deletions were predicted from paired-end spans larger than a specified cutoff D; simple insertions had a span < cutoff I; inversions are seen when ends map to the genome at different relative orientations; other types of insertions (defined in the text as mated and unmated) were detected with evidence of sequence integration from a distal locus.

Table 1. Validation of SVs identified by PEM. Array-CGH experiments were scored for indels in NA15510 not shared with NA18505. An additional 88 SV breakpoint junctions were deduced from the Celera assembly (table S1). Totals are underlined. Genomewide estimates of SVs are 761 for NA15510 and 887 for NA18505.

	Total SVs	Intersection with SVs/CNVs in DGV	SVs confirmed by Celera assembly	Array-CGH	Fiber-Fish (no. of validated inversions indicated)	PCR spanning breakpoint junctions	SV events with sequenced breakpoint junctions
			Female of presumably European ancestry (NA15510)				
SVs detected by PEM	472	278	104	31	2	157	52
SV indels	422	249	95	31		132	51
Inversions	50	29	9		2	25	1
			Female of African ancestry (NA18505)				
SVs detected by PEM	825	495	103		2	354	61
SV indels	753	454	97			328	59
Inversions	72	41	6		2	26	2

locus; one of the two expected breakpoints remained undetected. Unless stated otherwise, we treated insertions and deletions as "SV indels," because a deletion in one individual corresponds to an insertion in the other. These events can be distinguished with additional analyses (see below).

For all rearrangement types (i to v), we required that SVs were supported by at least two independent paired-end reads to eliminate false-

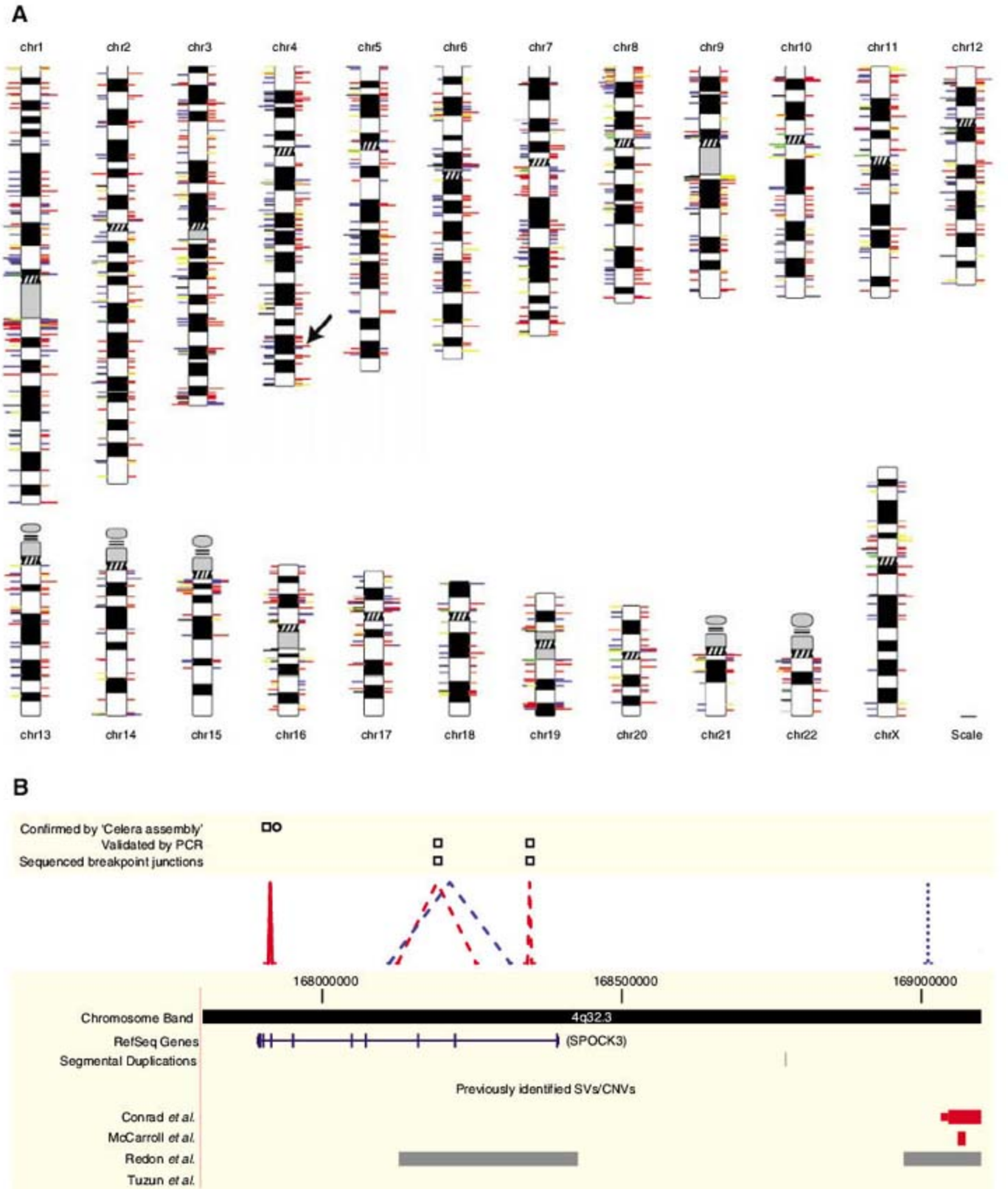


Fig. 2. SVs identified in two humans. **(A)** SVs mapped onto chromosomal ideograms (*12*). Right side: Red, deletion; blue, insertion; and yellow, inversion. Double length indicates SVs observed in both individuals. Left side: Log-scale size of an event (events ≥1 Mb are drawn at same length, corresponding to the maximum length of a line); unmated insertions [i.e., events lacking a predicted breakpoint and thus size information (*12*)] and simple insertions (*12*) are depicted with 1-kb lines; line colors indicate repetitive sequences in ±3-kb window of the predicted breakpoint junction (*12*): red, SDs; blue, LINEs; yellow, LTRs; green, satellites; black, two or more repetitive elements with equal frequency; gray, no repeat association. The arrow indicates the region in (B). A high-resolution image of this figure is available as fig. S1. **(B)** Amplified view of chromosome 4 region. SVs in NA18505 are indicated with dashed lines (validation: squares); NA15510, dotted lines (validation: circle). SVs shared between individuals are solid lines. Colors are as in (A).

positives that may arise from rare chimerical constructs that can form during the ligation reaction (*12*). This approach identifies deletions, inversions, mated insertions, and unmated insertions that are ~3 kb or larger, as well as simple insertions 2 to 3 kb in size. From two or more paired-end sequences per SV, we obtained an average breakpoint resolution of 644 base pairs (bp) (*12*), a range that facilitates the validation of SVs by polymerase chain reaction (PCR).

PEM detection of SVs in the human genome. We applied PEM to map SVs in the genomes of two individuals: a female (NA15510) in which 297 SV events had been mapped with FPES (*12*) and a second female (NA18505; Yoruba, Ibadan, from Nigeria) previously analyzed in the international HapMap project (*14*). The ancestry of NA15510 is unknown, however, the individual appears to be of European descent as described below. We sequenced over 10 million (NA15510) and 21 million (NA18505) paired ends, yielding effective coverages of 2.1- and 4.3-fold relative to the six billion–base pair diploid genome (*12*) to identify ~62% and 93% of the SVs, respectively (*12*). We identified 1175 SV indels (853 deletions, 322 insertions, i.e., 39 simple, 82 mated, 201 unmated) and 122 inversions, for a total of 1297 SV events (Table 1 and tables S1 and S2). For 20% of these events, only one out of two expected breakpoint junctions were identified (particularly in the European sample, which lacks saturation). Extrapolating to full coverage, we predict 761 and 887 SV events relative to the reference genome for NA15510 and NA18505, respectively, at this level of resolution. SVs were distributed throughout the genome with a number of hotspots (Fig. 2), such as an 8-megabase (Mb) region at 22q11.2 containing 13 SVs and an 18-Mb region at 7q11 containing 29 SVs. Both regions are involved in relatively frequent genomic disorders (velocardiofacial syndrome and Williams-Beuren syndrome, respectively), and SVs in healthy individuals at those loci were previously observed at lower resolution [e.g., (*2*)].

We compared the SVs identified in NA15510 to those in NA18505 and found that nearly half (45%) of the predicted SVs were shared between them (table S3): that is, 43% of the deletions, 52% of the insertions, and 43% of the inversions (*12*). Thus, a considerable fraction of the SV events occur commonly in the population and are presumably ancient. It is also possible that "common" SVs are due to errors in the human reference sequence. However, this is likely to be rare, as 18 of 19 cases we tested by PCR contained the reference sequence in one or more DNA samples. Thus, many of the detected events are bona fide SVs and likely to occur commonly in humans.

We were able to confirm 41% of all deletion and inversion events predicted in (*3*) for NA15510. Because only 62% of NA15510 is covered in our study, extrapolation to full coverage predicts that PEM would identify ~65% of all SVs predicted in (*3*), including 70% of the

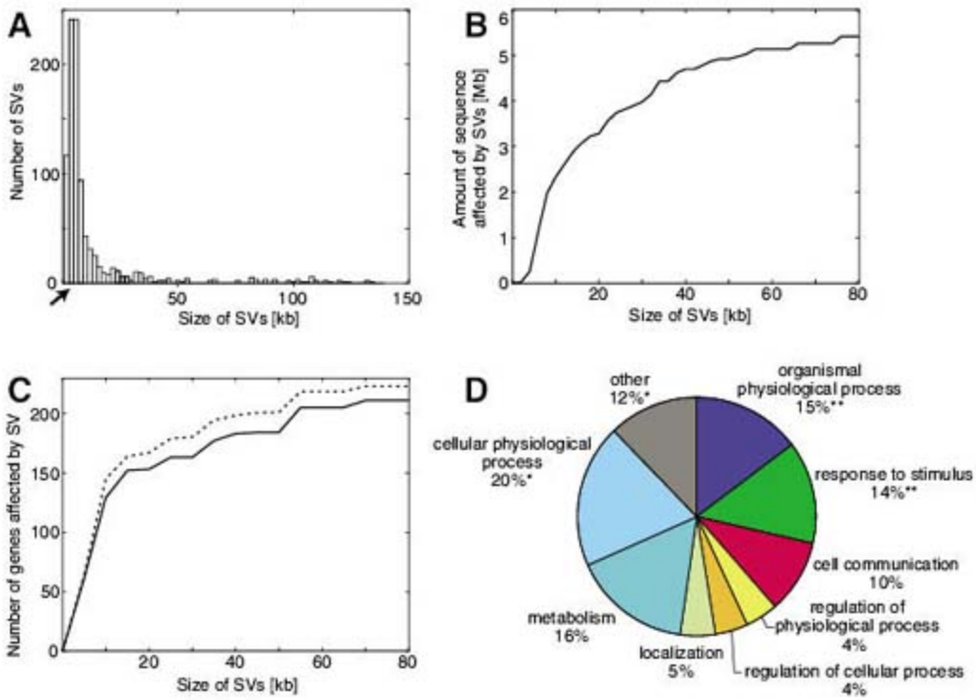


Fig. 3. SV size distribution, sequence coverage, genes, and distribution of gene categories. **(A)** Size distribution of SVs (NA15510 and NA18505 combined). Arrow indicates the lower size cutoff for deletions. **(B)** Cumulative number of base pairs affected by SVs in relation to SV size (NA18505 only). **(C)** Solid line indicates cumulative number of RefSeq genes intersecting with SVs in relation to SV size (NA18505 only). Dashed line, randomly shuffled SV locations within the local genomic context (±50-kb window) exhibit an increase in gene overlap. **(D)** Enrichment or depletion of GO (annotation level 3) biological processes for genes intersecting with SVs (NA15510 and NA18505 combined). Annotations represented by <10 genes are designated "other" and are gray. **Significant enrichment in genes belonging to a category ($P < 1e^{-14}$) (*12*); *significant depletion ($P < 0.001$).

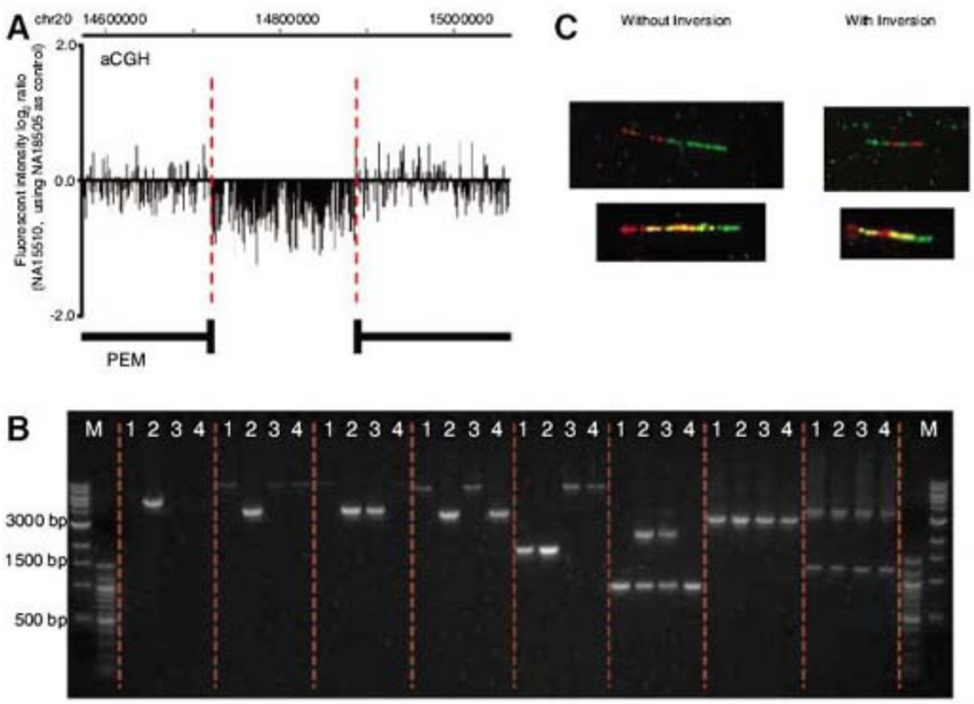


Fig. 4. Validation of SVs. **(A)** A 170-kb deletion detected with both array-CGH and PEM. **(B)** PCR products validating SVs as originally predicted from NA18505 (lane 2). Lanes 1 to 4 use DNAs from NA15510, NA18505, NA11997 (HapMap CEU, cell lines derived from 30 trios of European descent), and NA18614 (HapMap CHB, Han Chinese from Beijing), respectively. Primer sequences can be found in table S6. **(C)** Fiber-FISH validation of heterozygous inversions in NA18505. The inversion in the upper panel was independently validated in NA15510. Alternating patterns of fluorescent labels from adjacent probes indicate genomic rearrangement.

deletions. False-positives may account for some of the discrepancies between studies, although 83 and 97% of predicted events were confirmed by (*3*) and us, respectively (see below). It is also possible that these two studies have different, conservative thresholds [see (*3*) and (*12*)], reducing the identification of true events. Regardless, PEM identified an additional 407 SVs (377 SV indels, 30 inversions) in NA15510 not previously detected, including many events <8 kb and also larger variants. Similarly, more SVs were detected in NA18505 than those previously identified at lower resolution (*4*), with an additional 813 SVs identified and fine-mapped.

The majority of SVs detected by PEM were small (Fig. 3). About 65% of all SVs were <10 kb and 30% were <5 kb; however, 15% of all predicted SVs were larger than 100 kb and events up to megabase level in size were predicted; size distributions were similar for NA15510 and NA18505. In addition, the size and extent of SVs found indicates that healthy individuals differ by several megabases of nucleotide sequence (Fig. 3B and table S1). We analyzed the fraction of heterozygous and homozygous SVs by PCR analysis (for both NA15510 and NA18505), and we searched for the allele represented in the human reference genome with paired-end sequences [for NA18505 (*12*)]. Our results confirmed a previous study (*3*) and revealed that 23% and 15 to 20% of the SVs in NA15510 and NA18505, respectively, are homozygous (*12*).

SV validation. To validate PEM-SVs, we performed PCR analysis on 40 randomly chosen samples with five sets of primers spanning predicted breakpoint junctions (*12*). Of 34 SVs that could be scored, 33 (97%) yielded a single, clear PCR band at the expected size range (*12*). SVs were also confirmed and validated with five additional approaches: (i) comparison with SVs in DGV (*2*), (ii) comparison with an alternative human genome assembly ("Celera assembly"),

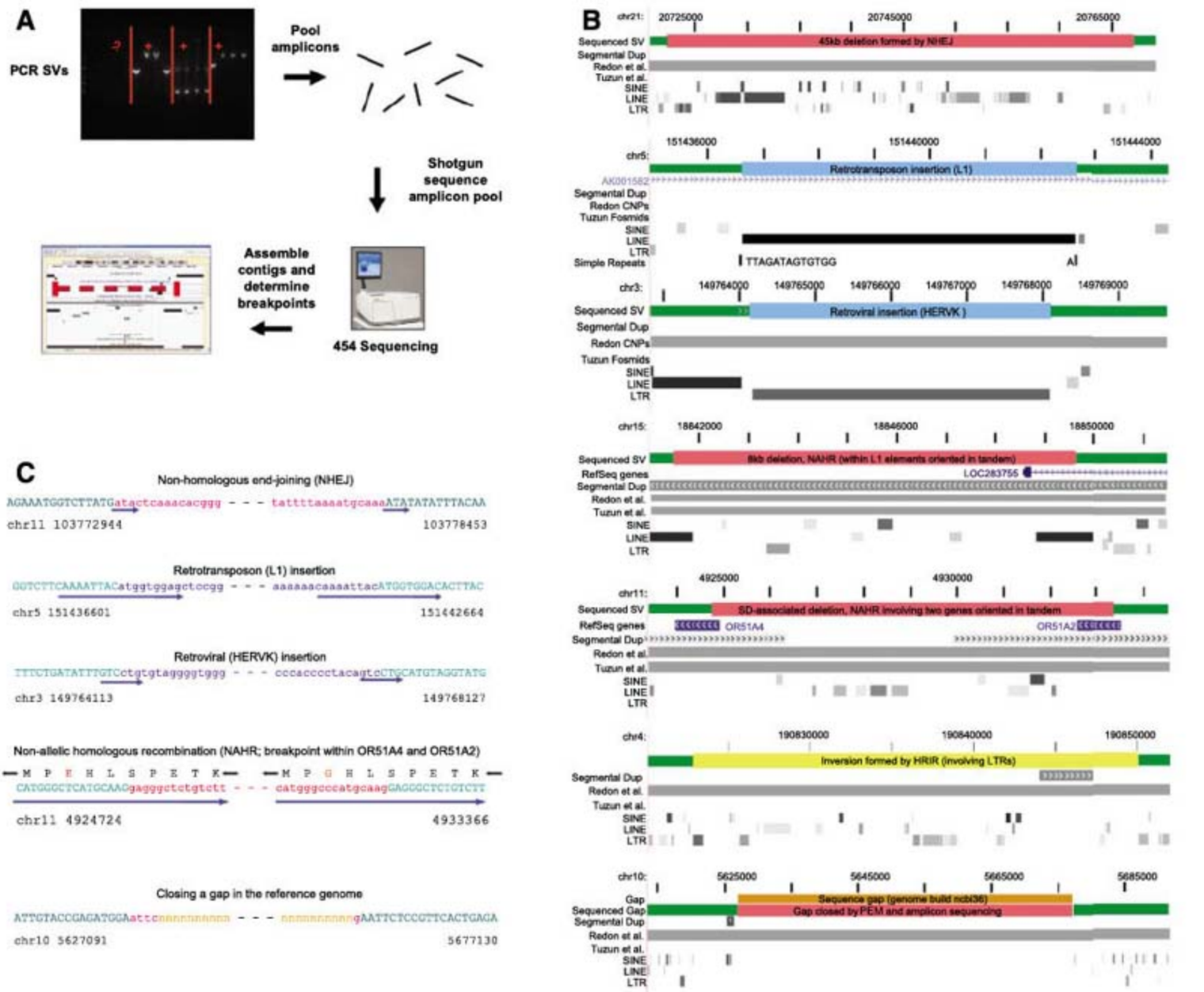


Fig. 5. Sequencing and analysis of SV breakpoint junctions. (**A**) PCR fragments spanning SVs were pooled and sequenced; breakpoints were determined from assembled contigs or ≥2 sequencing reads. (**B**) Representative sequenced SVs shown in relation to previous SV and/or CNV assignments [earlier SV and CNV assignments often extend outside of the depicted regions (*3*, *4*)]. From top to bottom: SVs resulting from NHEJ, L1 retrotransposition, HERVK (retrovirus) insertion, (nonallelic) homologous recombination, gap closure (blue, insertions; red, deletions; orange, sequence gap; and yellow, inversions). Note that some SVs affect annotated genes. (**C**) Example breakpoint sequences (*12*). Upper case and green letters are for unaltered sequence; lower case for SV indel; solid arrows show microhomologies (indicative of NHEJ), duplication of target sequences (at retrotransposon or retrovirus insertion sites), and long stretches of sequence identity (*12*) (indicative of homologous recombination). Note that the fourth sequence (from top to bottom) shows an OR gene fusion in the main reading frame (breakpoints occurred in the long stretch of sequence identity).

(iii) DNA microarray–based high-resolution comparative genome hybridization (array-CGH) (*15*, *16*), (iv) fiber-based, fluorescence in situ hybridization (fiber-FISH), and (v) a one-pass PCR assay spanning SV breakpoint junctions.

We found that 59 and 60%, respectively, of NA15510 and NA18505 SVs intersected with SVs represented in DGV (Table 1); the figures increase to 91 and 90%, respectively, for variants in the range 50 to 500 kb (*12*). Because the resolution of most SVs in DGV is low (>50 kb) (*17*), it is unclear whether the overlapping variants correspond to the same event. Comparison with the Celera assembly confirmed 104 (22%) and 103 (12%) of the NA15510 and NA18505 SVs, respectively (*12*). A higher fraction of events is probably shared with the Celera assembly, as many (>200) SV regions aligned poorly or coincided with gaps in the Celera assembly and were thus excluded from this analysis (*12*). The observation that a higher fraction of SVs is shared between NA15510 and the Celera assembly (which is primarily derived from a donor of European ancestry) indicates that NA15510 is of European origin.

Array-CGH experiments compared NA15510 DNA to NA18505 DNA with a set of eight oligonucleotide tiling arrays covering nonrepetitive regions of the genome. Of 48 NA15510-specific indels represented by at least 10 probes on the array [our detection limit (*12*)], 31 (65%) were validated by array-CGH (see Fig. 4 and table S1). The imperfect overlap may be because either some NA15510 SVs intersect with SVs in NA18505 and thus may not yield good array signals and/or array-CGH misses a portion of true-positive events (*4*).

For four inversions, not reported previously in DGV (*2*), we performed fiber-FISH on stretched DNA and located PEM-identified inversion breakpoints at the correct position for three (Fig. 4 and table S1). We were unable to detect the fourth inversion, presumably because its size (≤4 kb) is below our detection limit for fiber-FISH.

In order to validate SVs for downstream sequence analyses, we further analyzed 261 SVs predicted in NA15510 and 616 predicted in NA18505 in a one-pass PCR test, focusing primarily on SVs not represented in the Celera assembly. For 249 SVs, DNA from a total of four individuals was analyzed (Fig. 4): NA15510, NA18505, NA11997 (European ancestry), and NA18614 (Asian). As a result, 58% of the predicted SVs were validated by PCR in one or more individuals, including the sample in which the SV was originally identified. For the tests performed on all four individuals, 89% shared SVs among two or more individuals and 48% shared SVs in all four individuals. We also examined segregation patterns of five SVs in parent-offspring trios and observed Mendelian segregation patterns of SVs in nine meioses (*12*). Thus, their presence in multiple individuals and their Mendelian segregation patterns indicate that the majority of SVs are genetically stable and unlikely to have formed de novo or in the cultured cells that were analyzed (*4*). Altogether, 551 unique SVs were validated by array-CGH, fiber-FISH, the Celera assembly, and/or PCR (table S1).

Overlap of SVs with genes and functional elements. We compared the locations of predicted SVs smaller than 100 kb [for which assigned sizes had high confidence (*12*)] with annotated genes and functional elements. We found that many (17%) of the SVs in both individuals may directly affect gene function by removing exons or fusing annotated protein-coding genes (40 RefSeq genes), by being positioned in introns (243 genes), or by altering gene copy number or orientation (32 genes). The fraction of SVs affecting genes is slightly less than that expected by chance (Fig. 3C), which suggests selective constraint against SVs (*4*). We also analyzed protein-coding genes by their gene ontology (GO) functional classes. Consistent with previous observations (*1*–*4*), we found genes involved in organismal physiological processes (e.g., immunity, and cell-cell signaling; both with $P < 1e^{-14}$; hypergeometric test; Bonferroni correction) to be enriched with SVs (*12*), whereas genes involved in cellular physiological processes were depleted ($P < 0.001$) (Fig. 3D). Genes encoding proteins involved in interactions with the environment such as immune response, perception of smell, and perception of chemical stimuli were particularly likely to harbor SVs (*12*). Retrovirus- and transposition-related proteins also contain more SVs than expected by chance.

Genomewide analysis of SVs and associated breakpoints. To study SV formation, we determined the sequences surrounding breakpoint junctions with a new high-throughput approach (*12*). PCR products containing breakpoints were pooled and sequenced with 454 technology (*13*), and contigs were assembled (Fig. 5A). Breakpoints for a nonredundant set of 114 SVs were deduced with either a high-quality contig or at least two separate 454 reads (table S1). This method was most successful for SVs with breakpoints in regions that either have nonidentical DNA sequences or share short (<200-bp) identity at the junctions. The sequence data also allowed us to identify 344 putative SNPs located adjacent to the sequenced SVs (*12*), which may serve as future predictors for the SVs (table S4).

The 114 sequenced SVs included events confirmed by the Celera assembly. Manual inspection of sequence alignments in 14 cases indicated that all 14 corresponded to the same SVs evident in the Celera assembly (*12*). We therefore included in our analyses an additional 88 (nonredundant) SVs confirmed by the Celera assembly for which breakpoints could be assigned with high confidence, which yielded a total of 202 SVs identified by PEM with determined breakpoint junctions (188 SV indels and 14 inversions). The types of events observed from sequenced SVs were similar to those deduced from the Celera assembly.

We initially examined the association of breakpoint junctions with elements in the human genome. Several studies [e.g., see (*10*)] have suggested an association of SVs with segmental duplications (SDs); following the analysis scheme in (*18*), we find that 28 of 202 SVs have at least one breakpoint that directly intersects an SD [~2.6-fold enrichment over the genomic background, $P < 0.0001$ from permutations (*12*)]. Furthermore, many SVs occurred in short to medium-sized repetitive elements [30 for *Alu* SINEs (short interspersed nuclear elements), 74 for L1 LINEs (long interspersed nuclear elements), 3 for L2 LINEs, and 30 for LTRs (long terminal repeats)]. Out of the latter, L1 elements are significantly enriched (with $P < 0.01$), whereas L2 elements appear significantly depleted ($P < 0.0001$). Finally, *Alu* elements are not significantly enriched near SVs, despite previous reports (*19*).

Mechanisms of SV formation and effects on genes. Detailed manual analysis of the breakpoint junctions of SV indels revealed likely mechanisms as to how most SVs arose (see Fig. 5, B and C) and, in most cases, allowed us to distinguish insertion and deletion events. For example, entire L1 elements with poly(A) tails near the breakpoint junctions are inferred to be insertion events; recombination between homologous regions resulting in sequence loss indicates deletions. Insertion and deletion events can be further confirmed by comparison with other primate sequences (*12*).

Most SV indels originated from nonhomologous end-joining (NHEJ) (56%) and retrotransposition events (30%). NHEJ (*20*), in which breakpoint junctions were flanked by nonhomologous regions [except for short stretches of identical sequence ("microhomology"), typically <5 bp, that flank the junction] was prevalent even among large SVs (Fig. 4A and table S1) and in regions with large SDs. Most (90% of) retrotransposition events were due to L1 elements, although a small fraction (8%) corresponded to SVA [a composite element that was derived from three other repeats: short interspersed nuclear element-R, VNTR (variable number of tandem repeats), and *Alu*] elements (*21*). We also observed one instance of retroposition by an endogenous retrovirus, despite conflicting reports suggesting that these are not active or move infrequently in humans (*21*). Our finding indicates that these elements have been mobile in relatively recent human history (*22*). DNA transposition events (*21*) were not observed.

SVs have been found to be associated with duplicated regions, which suggests that many form by nonallelic homologous recombination (NAHR). Even though SVs and SDs are strongly associated, relatively few events (14% of all SV indels) are likely mediated by NAHR [recognizable through homologous regions flanking the breakpoint junctions (*12*)]. NAHR was rare even for large SVs as only 2 of 21 SV indels >20 kb in size originated from NAHR. Of these, 18 were formed through NHEJ, and for one, the mechanism was not assigned. NAHR events were located in: (i) highly repetitive elements: L1

elements (four cases), LTR elements (five cases), SINEs (six cases) and simple sequences (two cases), and (ii) high complexity regions: SDs (five cases) and unique DNA (five cases). As an interesting example of the latter, we observed a fusion involving the protein-coding regions of two olfactory-receptor (OR) genes, *OR51A4* and *OR51A2*, resulting in a new gene predicted to encode a protein identical to OR51A4, with upstream regions from *OR51A2* (Fig. 5, B and C). *OR51A4* and *OR51A2* are found in the rhesus monkey; their presence confirms that the "ancestral" region contains both genes and that SV formation involved a recent gene-fusion event. We suggest that deviation in gene content for the large OR gene family may lead to diversity of olfactory perception in the human population.

In addition to NHEJ, retrotransposition, and NAHR, other events may have occurred or could not be assigned. In four cases, simple sequence DNA was present at the breakpoint junctions; NAHR or other mechanisms may be involved in their formation (*23*). Four cases were unassigned, and two sequenced SVs closed gaps in the human reference sequence (see, e.g., Fig. 5, B and C).

We also analyzed 14 inversions. Four instances of homologous recombination between inverted repeats (HRIR) were observed; surprisingly, the remaining 10 inversions appeared to involve events that do not require homology. Overall, a large fraction of all of the SVs we sequenced (at least 57%) had one or both breakpoints in nonrepetitive sequence, indicating that high-complexity genomic regions are subject to structural variation.

Discussion. PEM enabled global detection of SVs at 3-kb resolution, and an average resolution of breakpoint assignment of 644 bp. We identified ~1300 SVs in two individuals, which suggests that humans may differ to a greater extent in SVs than in SNPs, when considering the total number of nucleotides affected. To date, most human genome–sequencing projects do not directly analyze SVs. Our study reveals that, given their high frequency, it will be essential to incorporate SV detection into human genome–sequencing projects (*24*). Overall, PEM is a cost-effective method both for improving genome assemblies and for revealing SVs present in the genome for a better understanding of human diversity.

PEM has several advantages over existing methods. First, PEM increases resolution of SV detection to the level of confirmation by PCR, and resolution can be further improved by more careful selection of evenly sized DNA fragments for circularization. Second, PEM does not require preparation of a DNA library that involves cloning. However, the short size of fragments (3 kb) used in this study hampers the detection of simple insertions >3 kb, although larger insertions can be detected by their mated ends. Similar to other SV detection methods, a limitation of PEM is that SVs in regions with multiple copies of highly similar and long (>3 kb) repeats are difficult to identify. Fortunately, although 45% of the human genome is composed of high–copy number repeat elements, these are often sufficiently divergent or short and can thus be distinguished by PEM. Additional refinements of PEM are also possible and will eventually allow detection of all SVs in the human genome.

References and Notes

1. J. Sebat *et al.*, *Science* **305**, 525 (2004).
2. A. J. Iafrate *et al.*, *Nat. Genet.* **36**, 949 (2004).
3. E. Tuzun *et al.*, *Nat. Genet.* **37**, 727 (2005).
4. R. Redon *et al.*, *Nature* **444**, 444 (2006).
5. B. E. Stranger *et al.*, *Science* **315**, 848 (2007).
6. H. Stefansson *et al.*, *Nat. Genet.* **37**, 129 (2005).
7. E. Gonzalez *et al.*, *Science* **307**, 1434 (2005).
8. M. Fanciulli *et al.*, *Nat. Genet.* **39**, 721 (2007).
9. J. R. Lupski, P. Stankiewicz, *PLoS Genet* **1**, e49 (2005).
10. J. L. Freeman *et al.*, *Genome Res.* **16**, 949 (2006).
11. J. O. Korbel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 10110 (2007).
12. Materials and methods are available as supporting material on *Science* Online.
13. M. Margulies *et al.*, *Nature* **437**, 376 (2005).
14. The International HapMap Consortium, *Nature* **437**, 1299 (2005).
15. R. R. Selzer *et al.*, *Genes Chromosomes Cancer* **44**, 305 (2005).
16. A. E. Urban *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 4534 (2006).
17. B. P. Coe *et al.*, *Genomics* **89**, 647 (2007).
18. P. M. Kim *et al.*, in preparation, available at http://arxiv.org/abs/0709.4200v1.
19. J. A. Bailey, G. Liu, E. E. Eichler, *Am. J. Hum. Genet.* **73**, 823 (2003).
20. E. V. Linardopoulou *et al.*, *Nature* **437**, 94 (2005).
21. R. E. Mills, E. A. Bennett, R. C. Iskow, S. E. Devine, *Trends Genet.* **23**, 183 (2007).
22. R. Belshaw *et al.*, *J. Virol.* **79**, 12507 (2005).
23. A. Bacolla, R. D. Wells, *J. Biol. Chem.* **279**, 47411 (2004).
24. R. Khaja *et al.*, *Nat. Genet.* **38**, 1413 (2006).

25. We thank C. Turcotte, C. Celone, D. Riches, and 454 colleagues, and R. Bjornson at the Yale High Performance Computation Center (funded by NIH grant: RR19895-02) for technical support. Funding was provided by a Marie Curie Fellowship (J.O.K.), the Alexander von Humboldt Foundation (A.T.), the Wellcome Trust (N.P.C., M.E.H., J.C., and F.Y.), Roche Applied Science, and the NIH (Yale Center of Excellence in Genomic Science grant). Accessions can be found in table S5, and at http://sv.gersteinlab.org/.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1149504/DC1
Materials and Methods
Tables S1 to S6
Fig. S1
References

21 August 2007; accepted 13 September 2007
Published online 27 September 2007;
10.1126/science.1149504
Include this information when citing this paper.

REPORTS

Mussel-Inspired Surface Chemistry for Multifunctional Coatings

Haeshin Lee,[1] Shara M. Dellatore,[2] William M. Miller,[2,3] Phillip B. Messersmith[1,3,4]*

We report a method to form multifunctional polymer coatings through simple dip-coating of objects in an aqueous solution of dopamine. Inspired by the composition of adhesive proteins in mussels, we used dopamine self-polymerization to form thin, surface-adherent polydopamine films onto a wide range of inorganic and organic materials, including noble metals, oxides, polymers, semiconductors, and ceramics. Secondary reactions can be used to create a variety of ad-layers, including self-assembled monolayers through deposition of long-chain molecular building blocks, metal films by electroless metallization, and bioinert and bioactive surfaces via grafting of macromolecules.

Methods for chemical modification of bulk material surfaces play central roles in modern chemical, biological, and materials sciences, and in applied science, engineering, and technology (*1*–*4*). The existing toolbox for the functional modification of material surfaces includes methods such as self-assembled monolayer (SAM) formation, functionalized silanes, Langmuir-Blodgett deposition, layer-by-layer assembly, and genetically engineered surface-binding peptides (*5*–*9*). Although widely implemented in research, many available methods have limitations for widespread practical use; specific examples include the requirement for chemical specificity between interfacial modifiers and surfaces (e.g., alkanethiols on noble metals and silanes on oxides), the use of complex instrumentation and limitations of substrate size and shape (Langmuir-Blodgett deposition), or the need for multistep procedures for implementation (layer-by-layer assembly and genetically engineered surface-binding peptides).

Development of simple and versatile strategies for surface modification of multiple classes of materials has proven challenging, and few generalized methods for accomplishing this have been previously reported (*10*). Our approach is inspired by the adhesive proteins secreted by

mussels for attachment to wet surfaces (*11*). Mussels are promiscuous fouling organisms and have been shown to attach to virtually all types of inorganic and organic surfaces (*12*), including classically adhesion-resistant materials such as poly(tetrafluoroethylene) (PTFE) (Fig. 1A). Clues to mussels' adhesive versatility may lie in the amino acid composition of proteins found near the plaque-substrate interface (Fig. 1, B to D), which are rich in 3,4-dihydroxy-L-phenylalanine (DOPA) and lysine amino acids (*13*). In addition to participating in reactions leading to bulk solidification of the adhesive (*14*–*16*), DOPA forms strong covalent and noncovalent interactions with substrates (*17*).

DOPA and other catechol compounds perform well as binding agents for coating inorganic surfaces (*18*–*23*), including the electropolymerization of dopamine onto conducting electrodes (*24*); however, coating of organic surfaces has proven much more elusive. Hypothesizing that the coexistence of catechol (DOPA) and amine (lysine) groups may be crucial for achieving adhesion to a wide spectrum of materials, we identified dopamine as a small-molecule compound that contains both functionalities (Fig. 1E). We show that this simple structural mimic of *Mytilus edulis* foot protein 5 (Mefp-5) is a powerful building block for spontaneous deposition of thin polymer films on virtually any bulk material surface and that the deposited films are easily adapted for a wide variety of functional uses.

Simple immersion of substrates in a dilute aqueous solution of dopamine, buffered to a pH typical of marine environments (2 mg of dopamine per milliliter of 10 mM tris, pH 8.5), resulted in spontaneous deposition of a thin adherent polymer film (Fig. 1, F to H). Analysis by atomic force microscopy (AFM) indicated that the polymer film thickness was a function of the immersion time and reached a value of up to 50 nm after 24 hours (Fig. 1G). X-ray photoelectron spectroscopy (XPS) analysis of 25 diverse materials coated for 3 hours or more revealed the absence of signals specific to the substrate (solid red bars in Fig. 1H; see also fig. S1), indicating the formation of a polymer coating of 10 nm or more in thickness. Little variation in the atomic composition of the coating was found (blue circles in Fig. 1H), suggesting that the composition of the polymer coating was independent of the substrate composition. The nitrogen-to-carbon signal ratio (N/C) of 0.1 to 0.13 is similar to that of the theoretical value for dopamine (N/C = 0.125), implying that the coating is derived from dopamine polymerization. Evidence for dopamine polymerization was found through analysis of the modification solution by gel permeation chromatography (fig. S2) and of coated substrates by time-of-flight secondary ion mass spectrometry (TOF-SIMS) (fig. S3). Polymer was found both in solution and on the substrate, with TOF-SIMS clearly revealing signals corresponding to dihydroxyphenyl-containing polymer fragments. Although the exact polymerization mechanism is unknown at this time, it is likely to involve oxidation of the catechol to a quinone, followed by polymerization in a manner reminiscent of melanin formation, which occurs through polymerization of structurally similar compounds (*25*) (fig. S3).

The polydopamine coating is able to form on virtually all types of material surfaces (Fig. 1H): noble metals (Au, Ag, Pt, and Pd), metals with native oxide surfaces (Cu, stainless steel, and NiTi shape-memory alloy), oxides [TiO_2, noncrystalline SiO_2, crystalline SiO_2 (quartz) Al_2O_3, and Nb_2O_5], semiconductors (GaAs and Si_3N_4), ceramics [glass and hydroxyapatite (HAp)], and synthetic polymers {polystyrene (PS), polyethylene (PE), polycarbonate (PC), polyethylene

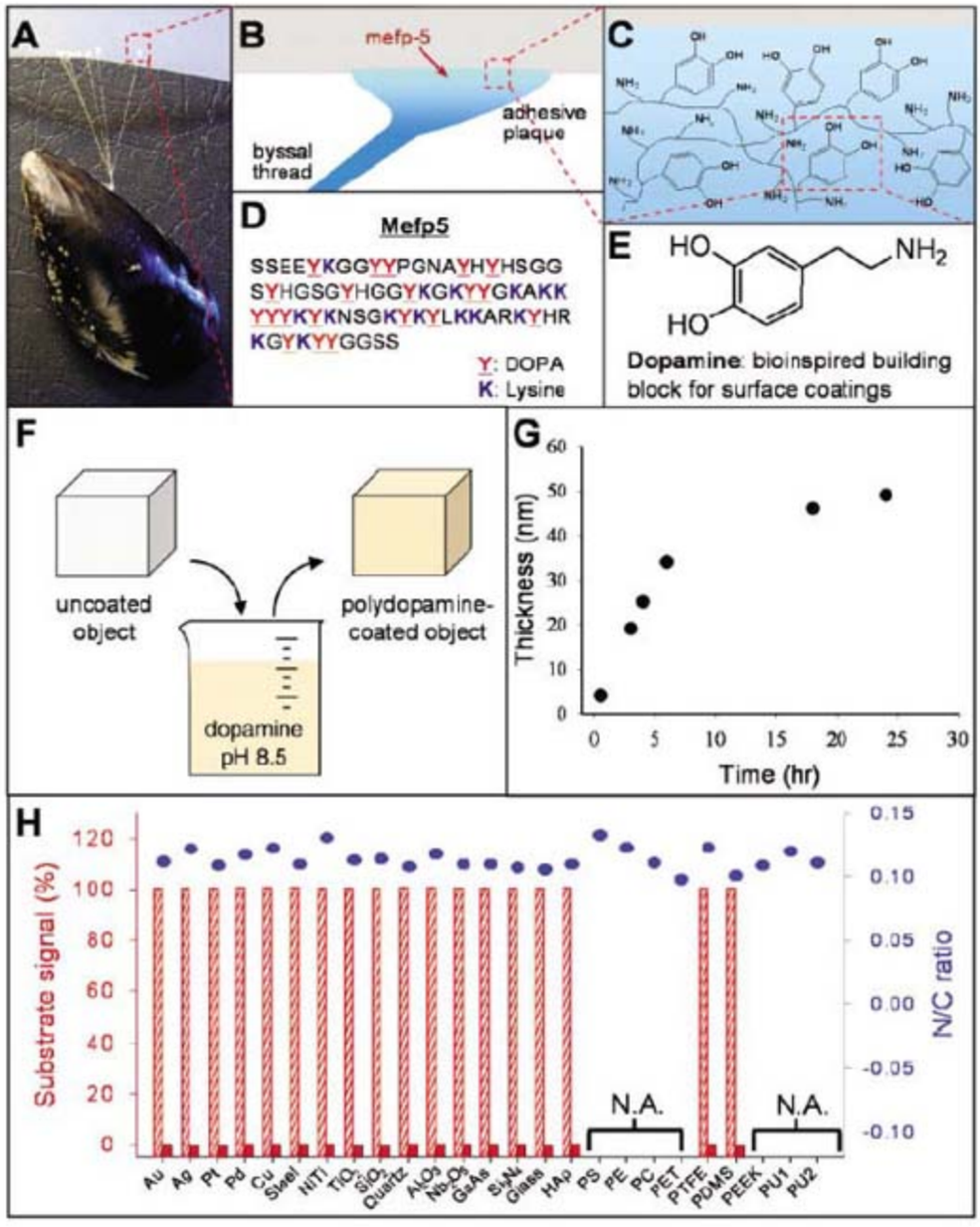


Fig. 1. (**A**) Photograph of a mussel attached to commercial PTFE. (**B** and **C**) Schematic illustrations of the interfacial location of Mefp-5 and a simplified molecular representation of characteristic amine and catechol groups. (**D**) The amino acid sequence of Mefp-5 (*13*, *34*). (**E**) Dopamine contains both amine and catechol functional groups found in Mefp-5 and was used as a molecular building block for polymer coatings. (**F**) A schematic illustration of thin film deposition of polydopamine by dip-coating an object in an alkaline dopamine solution. (**G**) Thickness evolution of polydopamine coating on Si as measured by AFM of patterned surfaces. (**H**) XPS characterization of 25 different polydopamine-coated surfaces. The bar graph represents the intensity of characteristic substrate signal before (hatched) and after (solid) coating by polydopamine. The intensity of the unmodified substrate signal is in each case normalized to 100%. Substrates with characteristic XPS signals indistinguishable from the polydopamine signal are marked by "N.A." The blue circles represent the N/C after polydopamine coating (details of XPS data analysis are available in fig. S1 and table S2).

[1]Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA. [2]Chemical and Biological Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA. [3]Institute for BioNanotechnology in Medicine, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA. [4]Materials Science and Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA.

*To whom correspondence should be addressed. E-mail: philm@northwestern.edu

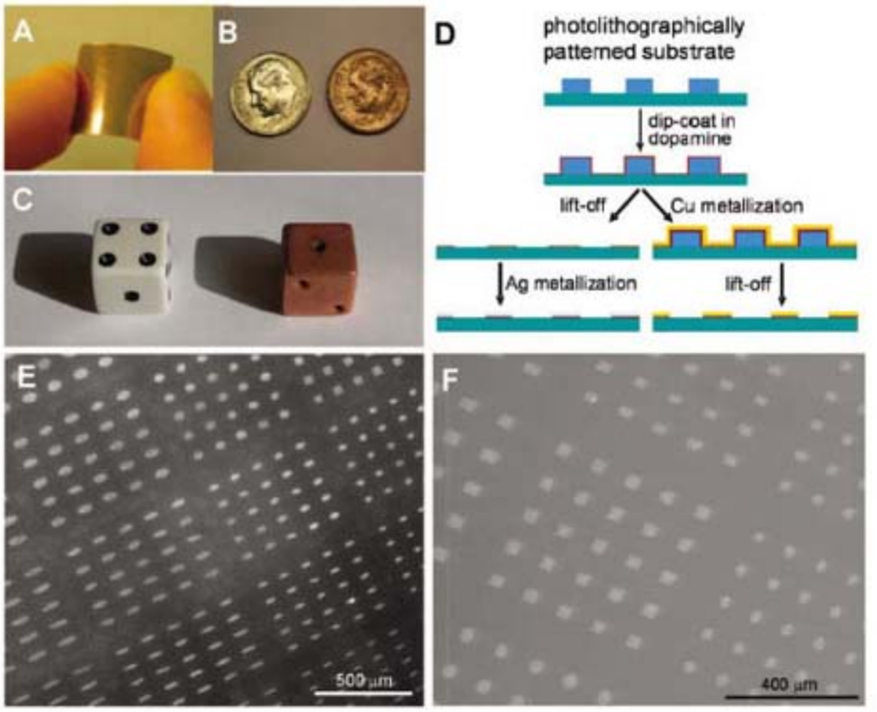


Fig. 2. Polydopamine-assisted electroless metallization of substrates. (**A** to **C**) Electroless copper deposition on polydopamine-coated nitrocellulose film (A), coin (B), and three-dimensional plastic object (C). (**D**) Schematic representation of electroless metallization of photoresist-patterned surfaces coated with polydopamine. Photoresist (blue) was removed before silver metallization (left) or after copper metallization (right). (**E** and **F**) Scanning electron microscopy images showing micropatterns of silver on Si (E) and copper on a glass substrate (F).

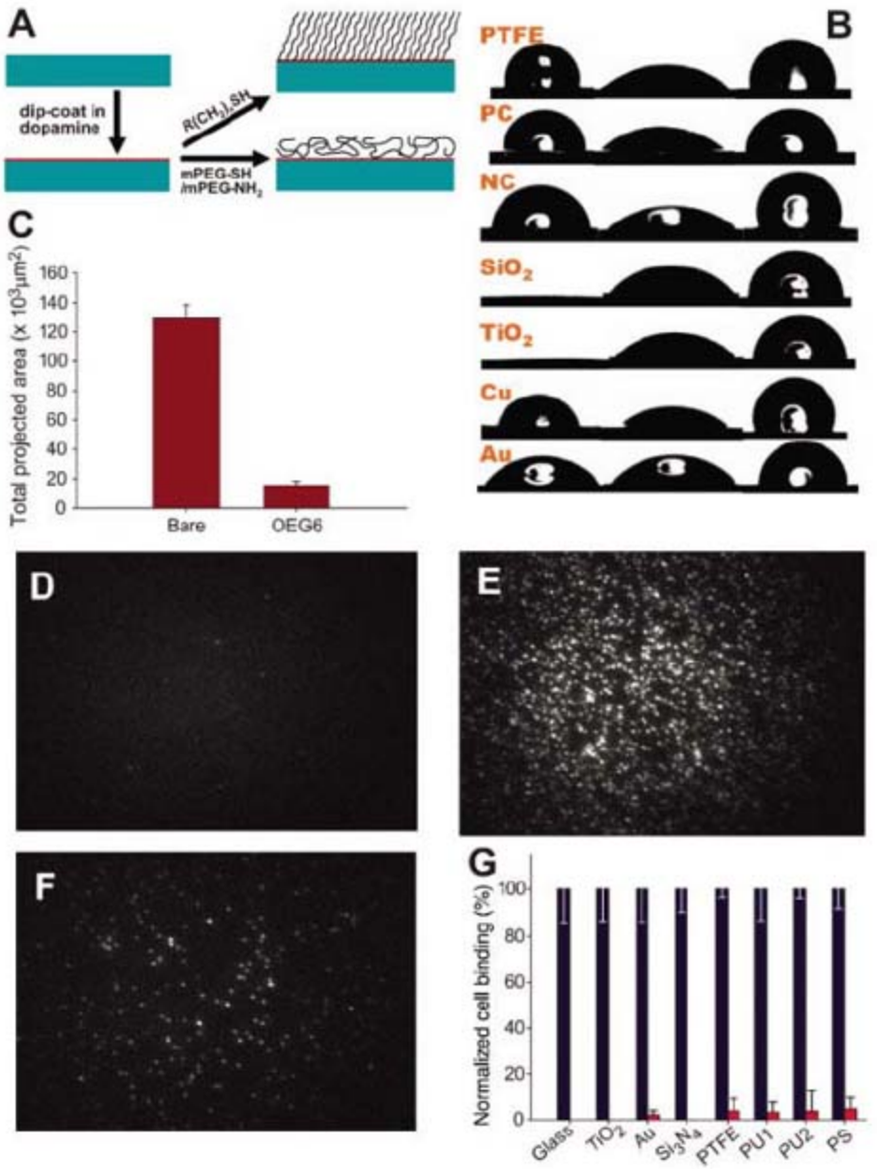


Fig. 3. Polydopamine-assisted grafting of various organic molecules. (**A**) Schematic illustration of alkanethiol monolayer (top right) and PEG polymer (bottom right) grafting on polydopamine-coated surfaces. (**B**) Pictures of water droplets on several unmodified (left), polydopamine-coated (middle), and alkanethiol-grafted (right) substrates. Substrates investigated include organic polymers [PTFE, PC, and nitrocellulose (NC)], metal oxides (SiO_2 and TiO_2), and noble metals (Cu and Au). Contact angle values are shown in table S1. (**C**) NIH 3T3 fibroblast cell adhesion to unmodified glass ("Bare") and OEG6-terminated alkanethiol monolayer formed on polydopamine-coated glass. Error bars indicate SD. (**D** to **F**) Total internal reflection fluorescence (TIRF) microscopy of Cy3-conjugated Enigma homolog protein adsorption to mPEG-NH_2–grafted polydopamine-coated glass (48-hour exposure to protein solution) (D), bare glass (30-min exposure) (E), and mPEG-silane immobilized on bare glass (48-hour exposure) (F). (**G**) NIH 3T3 fibroblast cell adhesion to bare surfaces (black) and to polydopamine-coated surfaces after grafting with mPEG-SH (red) (prenormalized data are available in table S3). Error bars indicate SD.

terephthalate (PET), PTFE, polydimethylsiloxane (PDMS), polyetheretherketone (PEEK), and polyurethanes [Carbothane (PU1) and Tecoflex (PU2)]}.

The polydopamine coating was found to be an extremely versatile platform for secondary reactions, leading to tailoring of the coatings for diverse functional uses. For example, the metal-binding ability of catechols (*26*) present in the polydopamine coating was exploited to deposit adherent and uniform metal coatings onto substrates by electroless metallization. This was demonstrated through deposition of silver and copper metal films via dip-coating of polydopamine-coated objects into silver nitrate and copper(II) chloride solutions, respectively (Fig. 2). Metal film deposition was confirmed by XPS and TOF-SIMS analysis, which demonstrated successful metal film deposition on several ceramic, polymer, and metal substrates: nitrocellulose, coinage metals, commercial plastics, Si_3N_4, glass, Au, TiO_2, SiO_2 PC, PS, PEEK, Nb_2O_5, Al_2O_3, and NiTi (figs. S4 and S5). Metal coatings were successfully applied in this manner to flexible polymer substrates and bulk objects with complex shapes (Fig. 2, A to C), as well as to flat surfaces in which the polydopamine coating had been patterned by means of standard photolithography techniques (Fig. 2, D to F). Unlike many other approaches to electroless metallization (*27*), the use of (immobilized) colloidal metal seed particles was unnecessary for spontaneous formation of adherent metal films. In the case of silver film deposition, the apparent reductive capacity of the polydopamine sublayer was sufficient to eliminate the need for addition of an exogenous reducing agent in the metal salt solution, implying oxidation of the underlying polydopamine layer.

Polydopamine coatings also support a variety of reactions with organic species for the creation of functional organic ad-layers. For example, under oxidizing conditions, catechols react with thiols and amines via Michael addition or Schiff base reactions (*14*, *28*) (fig. S3B). Thus, immersion of polydopamine-coated surfaces into a thiol- or amine-containing solution provided a convenient route to organic ad-layer deposition through thiol- and amine-catechol adduct formation (Fig. 3A). We demonstrated this approach for deposition of organic ad-layers in the form of alkanethiol monolayer, synthetic polymer, and biopolymer coatings.

A monolayer of alkanethiol was spontaneously formed through simple immersion of polydopamine-coated substrates (Fig. 3B). Monolayer formation on the polydopamine sublayer is believed to involve reaction between terminal thiol groups and the catechol/quinone groups of the polydopamine coating, in a manner analogous to the reaction between thiols and noble metal films in the formation of conventional SAMs. Alkanethiol monolayers formed by this approach are likely to contain defects but nevertheless appear to be functionally similar to conventionally formed SAMs. We therefore refer to these monolayers of alkanethiols as "pseudo-SAMs" (pSAMs). For example, spontaneous formation of pSAMs with the use of methyl-terminated alkanethiol (C12-SH) was suggested by water contact angles of greater than 100° (Fig. 3B and table S1) (*29*) and XPS spectra revealing the presence of sulfur in the modified surfaces (fig. S6). pSAMs were formed in this way on at least seven different materials, including several ceramics and polymers.

Through proper choice of secondary reactants, polydopamine coatings can be transformed into surfaces that have specific chemical properties, such as the suppression of nonspecific biological interactions or the promotion of specific ones (*23*, *24*). We first demonstrated this by formation of pSAMs from heterobifunctional molecular precursors on polydopamine-coated surfaces as described above. pSAMs terminated by oligo(ethylene glycol) (OEG6) were found to be largely resistant toward fibroblast cell attachment (Fig. 3C), behaving in a qualitatively similar fashion to nonfouling SAMs formed on gold (*30*).

Grafting of polymer ad-layers onto polydopamine coatings was accomplished through the use of thiol- or amine-functionalized polymers in the secondary reaction step, giving rise to bioresistant and/or biointeractive surfaces. For example, fouling-resistant surfaces were made by covalently grafting amine- or thiol-terminated methoxy-poly(ethylene glycol) [(mPEG-NH_2 or mPEG-SH) in 10 mM tris, pH 8.5, 50°C] to the polydopamine-coated surface (fig. S7). mPEG-NH_2–modified polydopamine-coated glass exhibited substantial reduction in nonspecific protein adsorption as compared with uncoated glass and also outperformed glass surfaces modified by a silane-terminated PEG in terms of fouling resistance after 2 days of continuous exposure to protein solution (Fig. 3, D to F). Similarly, mPEG-SH grafting onto a variety of polydopamine-coated

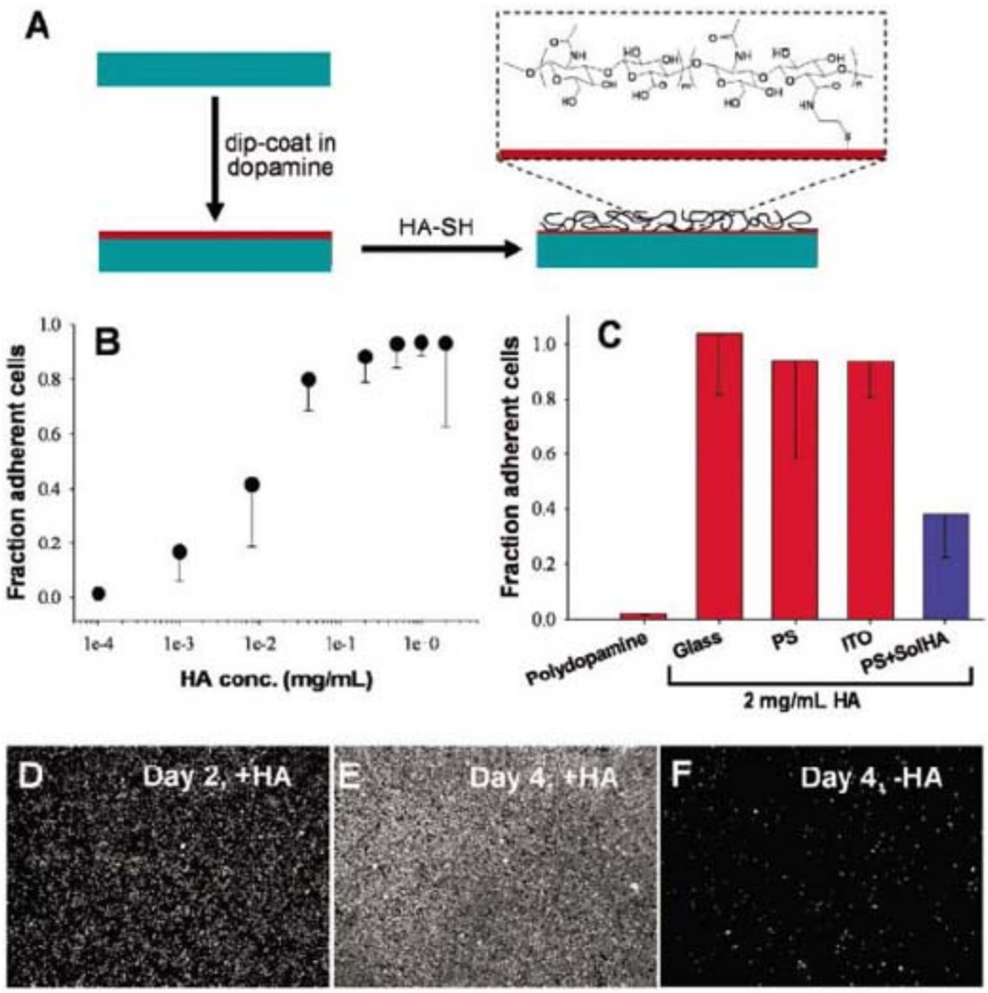


Fig. 4. Polydopamine-assisted grafting of a biomacromolecule for biospecific cell interaction. **(A)** Representative scheme for HA conjugation to polydopamine-coated surfaces. **(B)** Adhesion of M07e cells on polydopamine-coated PS increases with the HA solution concentration used during grafting. Error bars indicate SD. **(C)** Bioactive HA ad-layers were formed on polydopamine-coated glass, tissue-culture PS, and indium tin oxide (ITO), as demonstrated by attachment of M07e cells (red bars). Competition with soluble HA (blue bar) confirmed that cell adhesion was due to grafted HA. Error bars indicate SD. **(D to F)** Polydopamine-modified PS grafted with HA (0.5 mg of HA per milliliter of 10 mM tris, pH 8.0) retains bioactivity during long-term culture with M07e cells. Images taken after normal-force centrifugation show almost 100% attachment of expanding M07e cells at days 2 [2760 ± 390 cells/cm^2 (D)] and 4 [5940 ± 660 cells/cm^2 (E)]. In the absence of HA, the polydopamine-coated surface supported similar levels of M07e cell expansion at day 4 but did not support cell adhesion [610 ± 630 cells/cm^2 (F)].

substrates led to dramatic reduction of fibroblast cell attachment as compared with the unmodified substrates (Fig. 3G and table S3). The polydopamine coating itself was supportive of fibroblast cell adhesion at a level similar to that of bare substrates {for example, the total area of attached cells on 1.08 mm^2 of polydopamine-modified SiO_2 [$(46 \pm 1.4) \times 10^3$ μm^2] was similar to that of unmodified SiO_2 [$(55 \pm 8.6) \times 10^3$ μm^2]}, leading us to conclude that the observed decrease in cell adhesion was due to the grafted mPEG-SH.

Finally, we engineered polydopamine surfaces for specific biomolecular interactions by forming an ad-layer of the glycosaminoglycan hyaluronic acid (HA). HA/receptor interactions are important for physiological and pathophysiological processes, including angiogenesis, hematopoietic stem cell commitment and homing, and tumor metastasis (*31*, *32*). Partially thiolated HA (*33*) was grafted onto a variety of polydopamine-coated substrates (Fig. 4), and HA ad-layer bioactivity was measured via adhesion of the human megakaryocytic M07e cell line. Unlike fibroblasts, M07e cells did not adhere to polydopamine but did adhere to HA-grafted polydopamine surfaces in a dose-dependent manner (Fig. 4B). Together with decreased binding in the presence of soluble HA (Fig. 4C), these findings are consistent with expression of the HA receptor CD44 by M07e cells (fig. S8). Polydopamine and HA-grafted polydopamine surfaces were biocompatible, as evidenced by similar levels of M07e cell expansion as compared with cell expansion on tissue-culture PS surfaces, although only the HA-grafted polydopamine surfaces supported cell adhesion (Fig. 4, D to F, and fig. S9).

We introduced a facile approach to surface modification in which self-polymerization of dopamine produced an adherent polydopamine coating on a wide variety of materials. Polydopamine coatings can, in turn, serve as a versatile platform for secondary surface-mediated reactions, leading ultimately to metal, SAM, and grafted polymer coatings. This two-step method of surface modification is distinctive in its ease of application, use of simple ingredients and mild reaction conditions, applicability to many types of materials of complex shape, and capacity for multiple end-uses.

References and Notes

1. B. D. Ratner, A. S. Hoffman, Eds., *Biomaterials Science: An Introduction to Materials in Medicine* (Elsevier Academic, San Diego, CA, ed. 2, 2004).
2. J.-H. Ahn *et al.*, *Science* **314**, 1754 (2006).
3. P. Alivisatos, *Nat. Biotechnol.* **22**, 47 (2004).
4. R. Langer, *Science* **293**, 58 (2001).
5. J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo, G. M. Whitesides, *Chem. Rev.* **105**, 1103 (2005).
6. G. Decher, *Science* **277**, 1232 (1997).
7. G. Roberts, Ed. *Langmuir-Blodgett Films* (Plenum, New York, 1990).
8. S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara, A. M. Belcher, *Nature* **405**, 665 (2000).
9. C. Tamerler, M. Sarikaya, *Acta Biomater.* **3**, 289 (2007).
10. D. Y. Ryu, K. Shin, E. Drockenmuller, C. J. Hawker, T. P. Russell, *Science* **308**, 236 (2005).
11. J. H. Waite, M. L. Tanzer, *Science* **212**, 1038 (1981).
12. G. A. Young, D. J. Crisp, in *Adhesion*, K. W. Allen, Ed. (Applied Science, London, vol. 6, 1982).
13. J. H. Waite, X. X. Qin, *Biochemistry* **40**, 2887 (2001).
14. L. A. Burzio, J. H. Waite, *Biochemistry* **39**, 11147 (2000).
15. M. J. Sever, J. T. Weisser, J. Monahan, S. Srinivasan, J. J. Wilker, *Angew. Chem. Int. Ed.* **43**, 448 (2004).
16. M. Yu, J. Hwang, T. J. Deming, *J. Am. Chem. Soc.* **121**, 5825 (1999).
17. H. Lee, N. F. Scherer, P. B. Messersmith, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12999 (2006).
18. M. Yu, T. J. Deming, *Macromolecules* **31**, 4739 (1998).
19. J. L. Dalsin, B.-H. Hu, B. P. Lee, P. B. Messersmith, *J. Am. Chem. Soc.* **125**, 4253 (2003).
20. A. R. Statz, R. J. Meagher, A. E. Barron, P. B. Messersmith, *J. Am. Chem. Soc.* **127**, 7972 (2005).
21. T. Paunesku *et al.*, *Nat. Mater.* **2**, 343 (2003).
22. C. Xu *et al.*, *J. Am. Chem. Soc.* **126**, 9938 (2004).
23. S. Zürcher *et al.*, *J. Am. Chem. Soc.* **128**, 1064 (2006).
24. Y. Li, M. Liu, C. Xiang, Q. Xie, S. Yao, *Thin Solid Films* **497**, 270 (2006).
25. W. Montagna, G. Prota, J. A. Kenney Jr., *Black Skin: Structure and Function* (Academic Press, San Diego, CA, 1993).
26. C. G. Pierpont, C. W. Lange, *Prog. Inorg. Chem.* **41**, 331 (1994).
27. M. Charbonnier, M. Romand, G. Stremsdoerfer, A. Fares-Karam, *Recent Res. Dev. Macromol. Res.* **4**, 27 (1999).
28. M. J. LaVoie, B. L. Ostaszewski, A. Weihofen, M. G. Scholssmacher, D. J. Selkoe, *Nat. Med.* **11**, 1214 (2005).
29. P. E. Laibinis *et al.*, *J. Am. Chem. Soc.* **113**, 7152 (1991).
30. K. L. Prime, G. M. Whitesides, *J. Am. Chem. Soc.* **115**, 10714 (1993).
31. D. N. Haylock, S. K. Nilsson, *Regenerat. Med.* **1**, 437 (2006).
32. B. P. Toole, *Nat. Rev. Cancer* **4**, 528 (2004).
33. H. Lee, S. H. Choi, T. G. Park, *Macromolecules* **39**, 23 (2006).

34. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

35. This research was supported by NIH grants DE 14193 and HL 74151. The authors thank T. G. Park and H. Lee for donation of thiolated HA, N. F. Scherer and X. Qu for their generous discussion and technical assistance with TIRF microscopy, and K. Healy for photomask donation. This research used the NUANCE characterization facilities (Keck II, EPIC, and NIFTI) at Northwestern University.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/426/DC1
Materials and Methods
Figs. S1 to S10
Tables S1 to S3
References

2 July 2007; accepted 12 September 2007
10.1126/science.1147241

Structure of a Thiol Monolayer–Protected Gold Nanoparticle at 1.1 Å Resolution

Pablo D. Jadzinsky,[1,2]* Guillermo Calero,[1]* Christopher J. Ackerson,[1]† David A. Bushnell,[1] Roger D. Kornberg[1]‡

Structural information on nanometer-sized gold particles has been limited, due in part to the problem of preparing homogeneous material. Here we report the crystallization and x-ray structure determination of a *p*-mercaptobenzoic acid (p-MBA)–protected gold nanoparticle, which comprises 102 gold atoms and 44 p-MBAs. The central gold atoms are packed in a Marks decahedron, surrounded by additional layers of gold atoms in unanticipated geometries. The p-MBAs interact not only with the gold but also with one another, forming a rigid surface layer. The particles are chiral, with the two enantiomers alternating in the crystal lattice. The discrete nature of the particle may be explained by the closing of a 58-electron shell.

Nanometer-size metal particles are of fundamental interest for their chemical and quantum electronic properties and of practical interest for many potential applications (*1*, *2*). With the development of facile routes of synthesis (*3*), gold nanoparticles coated with surface thiol layers have been studied in most detail. The particles are typically heterogeneous as synthesized, and though their size distribution may be narrowed by fractionation or other means (*4*–*9*), no atomically monodisperse preparation has been reported, and no atomic structure has been obtained. Electron microscopy (EM) (*10*, *11*), powder x-ray diffraction (PXRD) (*12*), and theoretical studies have led to the idea of Marks decahedral (MD) and truncated octahedral geometries of the metal core, with crystalline packing and {111} faces (*13*). According to this idea, discrete core sizes represent "magic numbers" of gold atoms, arising from closed geometric shells (*14*). Alternatives of amorphous (*15*), molten, or quasimolten (*16*) cores have also been proposed. The structure of the surface thiol layer is similarly obscure. The nature of the gold-sulfur interaction (*17*), the fate of the sulfhydryl proton (*18*), and the conformation of the organic moiety all remain to be determined. The thiols are

[1]Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305, USA. [2]Department of Applied Physics, Stanford University, Stanford, CA 94305, USA.

*These authors contributed equally to this work.
†Present address: Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, USA.
†Present address: Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309, USA.
‡To whom correspondence should be addressed. E-mail: kornberg@stanford.edu

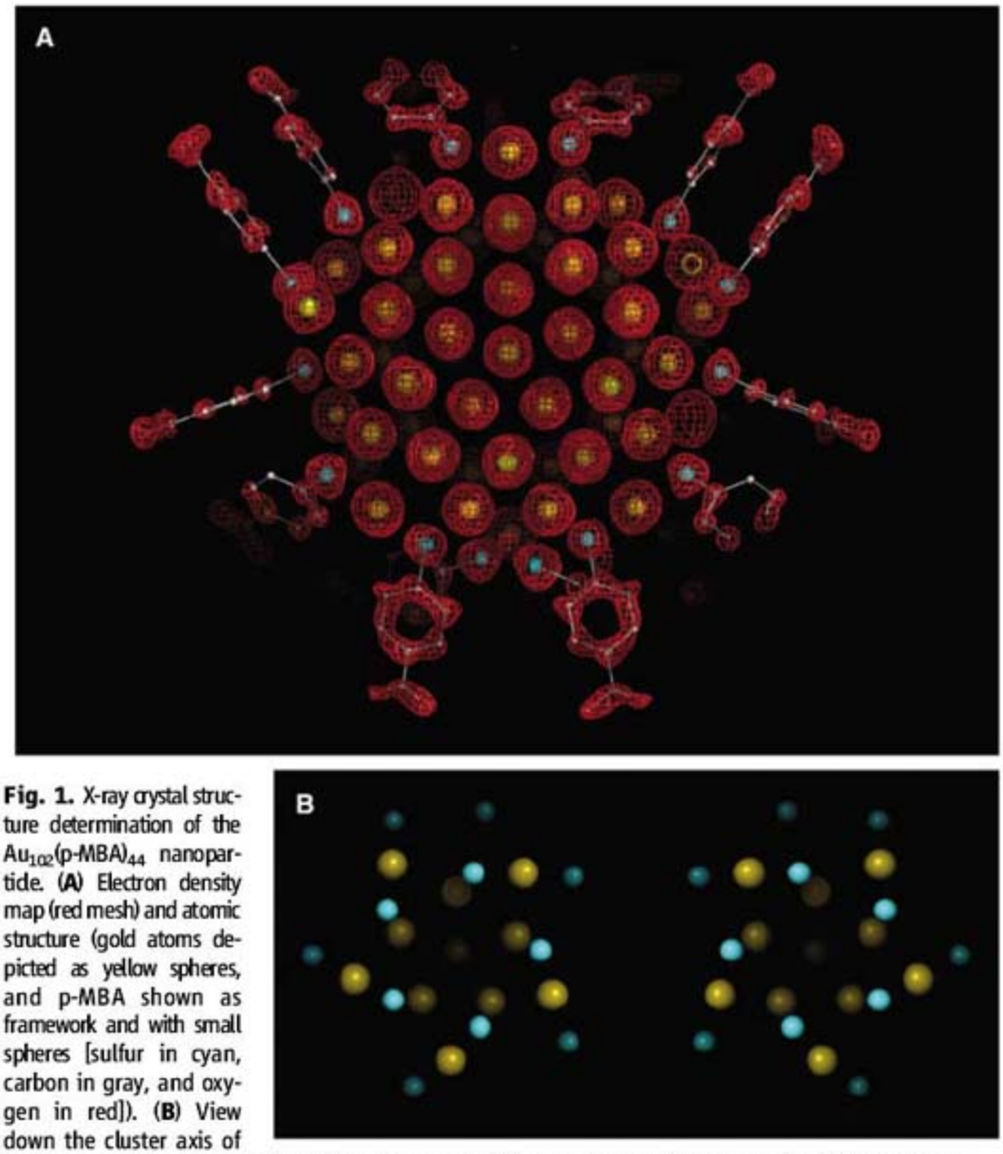


Fig. 1. X-ray crystal structure determination of the $Au_{102}(p\text{-}MBA)_{44}$ nanoparticle. **(A)** Electron density map (red mesh) and atomic structure (gold atoms depicted as yellow spheres, and p-MBA shown as framework and with small spheres [sulfur in cyan, carbon in gray, and oxygen in red]). **(B)** View down the cluster axis of the two enantiomeric particles. Color scheme as in (A), except only sulfur atoms of p-MBA are shown.

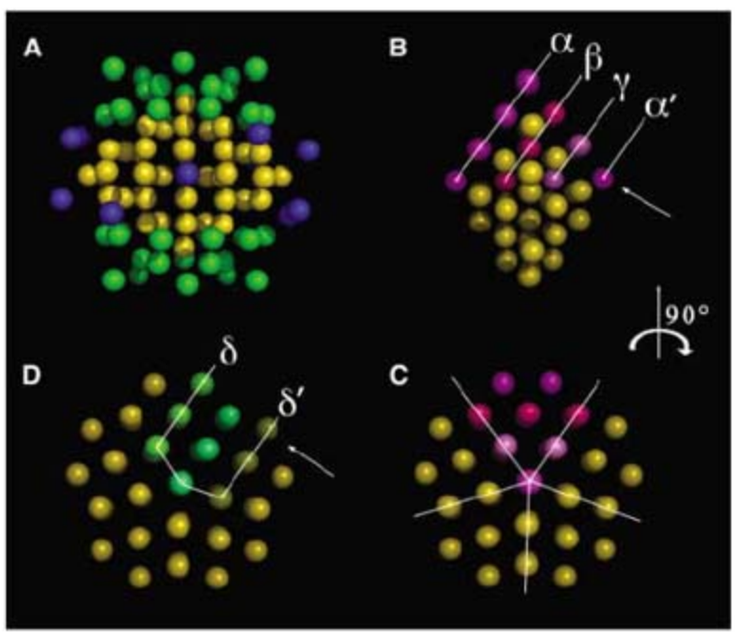


Fig. 2. Packing of gold atoms in the nanoparticle. **(A)** MD (2,1,2) in yellow, two 20-atom "caps" at the poles in green, and the 13-atom equatorial band in blue. **(B)** View of the MD with the axis horizontal, showing the fcc planes (α, β, γ, and α′). When viewed in the direction of the arrow, the fourth plane (α′) overlaps with the first (α), conforming to the definition of fcc planes. **(C)** View of the MD with the axis perpendicular to the page, showing how it may be regarded as five twinned crystals. **(D)** Same view as in (C) but showing hcp crystallites. When viewed in the direction of the arrow, the third plane (δ′) overlaps with the first (δ), conforming to the definition of hcp planes.

exchangeable and presumed to be mobile, further impeding structural analysis (*19*, *20*).

Through systematic variation of solution conditions for gold nanoparticle synthesis, we have obtained particles sufficiently uniform in size for the growth of large single crystals, opening the way to x-ray structure determination. We report here on x-ray analysis of a nanoparticle whose gold core and surface thiol structures differ markedly from what had been anticipated. The gold particles were coated with p-MBA and crystallized from a solution containing 40% methanol, 300 mM sodium chloride, and 100 mM sodium acetate, at pH 2.5 (*21*). The crystals were in the centrosymmetric space group $C2/c$, so diffraction showed no anomalous differences. We obtained initial phases using dispersive differences between data collected at the Au L_{III} edge and a low-energy remote, giving an electron density map that revealed 102 gold atoms and 44 p-MBAs. All electron density was accounted for by the structure (except for solvent water), so the clusters were entirely homogeneous and the numbers of gold atoms and p-MBAs were precise (Fig. 1A). The structure was refined at a resolution of 1.15 Å to R_{work} and R_{free} of 8.8 and 9.5%, respectively. The particles proved to be chiral, with half of an enantiomer in the asymmetric unit of the crystal (Fig. 1B and table S1).

Most gold-gold distances in the core lie in the range 2.8 to 3.1 Å (figs. S1 and S2). The core may be described as a 49-atom MD (2,1,2) with four atoms on the central axis, two 20-atom caps with C_5 symmetry on opposite poles (expanding to 89 the number of gold atoms with fivefold rotational symmetry), and a 13-atom band with no apparent symmetry on the equator (Fig. 2A). Alternatively, the MD may be described as five twinned face-centered cubic (fcc) or hexagonal close-packed (hcp) crystallites (Fig. 2, B to D) (*22*). All 102 gold atoms are found in environments with 12 nearest neighbors—fcc, hcp, icosahedral, or truncated decahedral—except that atoms near the surface lack from 1 to 10 neighbors. The 13 equatorial atoms occupy two different environments, which deviate slightly from local hcp or truncated decahedral (figs. S3 and S4). It is the number and geometry of the equatorial atoms that impart chirality to the core, and the deviations from local symmetry may reflect the interaction of the equatorial atoms with the p-MBA monolayer.

Gold atoms up to 5.5 Å from the center of the particle do not contact sulfur, those in a shell of radius 6.0 to 6.3 Å bind one sulfur, and those in a shell of radius 7.5 to 8.0 Å bind two sulfurs (Fig. 3A and fig. S5). All sulfur atoms lie in a shell of radius 8.3 ± 0.4 Å and bind in a bridge conformation (*23*) to two gold atoms; at least one of the gold atoms binds two sulfurs, forming a "staple" motif (Fig. 3, B and C). The gold-sulfur distance ranges from 2.2 to 2.6 Å (fig. S6). Gold-sulfur-gold angles are 80° to 115°, and sulfur-gold-sulfur angles are 155° to 175° (fig. S7). If the surface is taken as all gold atoms interacting with sulfur, then the coverage by p-MBA (thiol:gold ratio) is 70%, which is much higher than the val-

ues of 31 and 33% for benzenethiol (*24*) and alkanethiols (*17*) on Au(111) surfaces, reflecting the curvature of the nanoparticle surface.

The thiol monolayer is stabilized not only by gold-sulfur bonding but also by interactions between p-MBA molecules. These interactions are of three types: phenyl rings stacked on one another with the centers offset by the ring radius (Fig. 4A), phenyl rings interacting at right angles (T-stacking) (Fig. 4B), and sulfur interacting with a phenyl ring (Fig. 4C). Eighteen of the sulfur atoms are located over the face of a phenyl ring at a distance of about 3.55 ± 0.25 Å, similar to sulfur atoms engaged in aromatic-thiol π hydrogen bonding in proteins (*25*). Almost all sulfur atoms are also engaged in lone pair bonding to a phenyl edge (*25*). Most p-MBAs are linked through chains of such interactions extending from one pole of the nanoparticle to the other (Fig. 4D). This ordering of p-MBAs exemplifies the "self-assembly" of a thiol monolayer on a gold surface (*26*).

The chains of p-MBA interactions extending across the nanoparticle establish the chirality that is apparent from the view of the nanoparticle down the MD axis (Fig. 1B). Most sulfur atoms, bonded to gold atoms in two different shells and to a phenyl ring, are also chiral centers. One enantiomer has 22 sulfur centers with *R* configuration, 18 with *S* configuration, and 2 with no readily assigned chirality, because they are bonded to two gold atoms in the same shell.

The pairing of enantiomeric particles in the crystal demonstrates a surface complementarity of the particles (fig. S8). Interparticle interactions in the crystal thus reflect the chirality of the surface thiol layer. These interactions are of several types. Hydrogen bonding between carboxylic acids occurs at many crystal contacts (fig. S9), in some cases mediated by water molecules (*27*). Such interactions are frequent near the equator, where the phenyl rings extend outward from the particle surface. The p-MBAs from different nanoparticles interdigitate through phenyl-phenyl interactions, especially at the MD poles (fig. S10). Such interactions can explain the common finding that distances between neighboring clusters in two-dimensional gold particle arrays are less than twice the length of the fully extended thiol (*28*, *29*).

The very existence of a discrete $Au_{102}(p\text{-}MBA)_{44}$ nanoparticle is a notable finding from this work. Discrete sizes have been explained in the past by geometrical or electronic shell closing. The arrangement of gold atoms, with polar caps and an equatorial band, argues against geometrical shell closing. If, however, each gold atom ($5d^{10}6s^{1}$) contributes one valence electron, and 44 are engaged in bonding to sulfur, then 58 electrons remain, corresponding to a well-known filled shell. Indeed, a naked cluster in the gas phase containing 58 gold atoms shows exceptional stability (*30*–*32*).

There are several connections of the Au_{102} nanoparticle structure with previous work. First, structures of small gold, silver, and platinum clusters, and of large platinum-palladium clusters, include fivefold symmetry elements and, in one case, also include thiols bridging between pairs of gold atoms (*33*–*36*). Second, EM, PXRD, and theoretical studies of large gold clusters have given results that are consistent with a MD (*10*–*12*). Third, theoretical studies have raised the possibility of distinct gold-sulfur units capping a central gold core (*37*). Fourth, the fcc packing in the core, with a gold-gold distance of 2.8 to 3.1 Å, corresponds with the fcc packing in bulk metallic gold, with a gold-gold distance of 2.9 Å. Fifth, the staple motif, containing alternating gold and sulfur atoms (Fig. 3C), resembles the gold-thiol polymers believed to represent intermediates in the process of nanoparticle formation (*38*). Finally, circular dichroism measurements on gold nanoparticle preparations have shown chiro-optical activity (*39*).

We have screened 15 crystals derived from multiple gold nanoparticle preparations and obtained the same Au_{102} structure, so the unusual arrangement in the 13-atom equatorial band is a consistent result. Other nanoparticle preparations, however, which have also given rise to large single

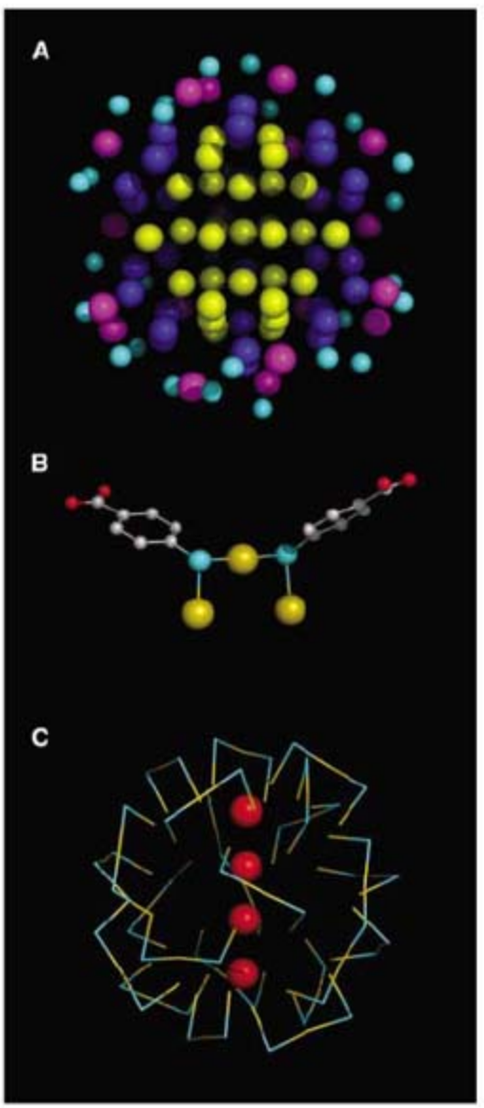


Fig. 3. Sulfur-gold interactions in the surface of the nanoparticle. **(A)** Successive shells of gold atoms interacting with zero (yellow), one (blue), or two (magenta) sulfur atoms. Sulfur atoms are cyan. **(B)** Example of two p-MBAs interacting with three gold atoms in a bridge conformation, here termed a staple motif. Gold atoms are yellow, sulfur atoms are cyan, oxygen atoms are red, and carbon atoms are gray. **(C)** Distribution of staple motifs in the surface of the nanoparticle. Staple motifs are depicted symbolically, with gold in yellow and sulfur in cyan. Only the gold atoms on the axis of the MD are shown (in red).

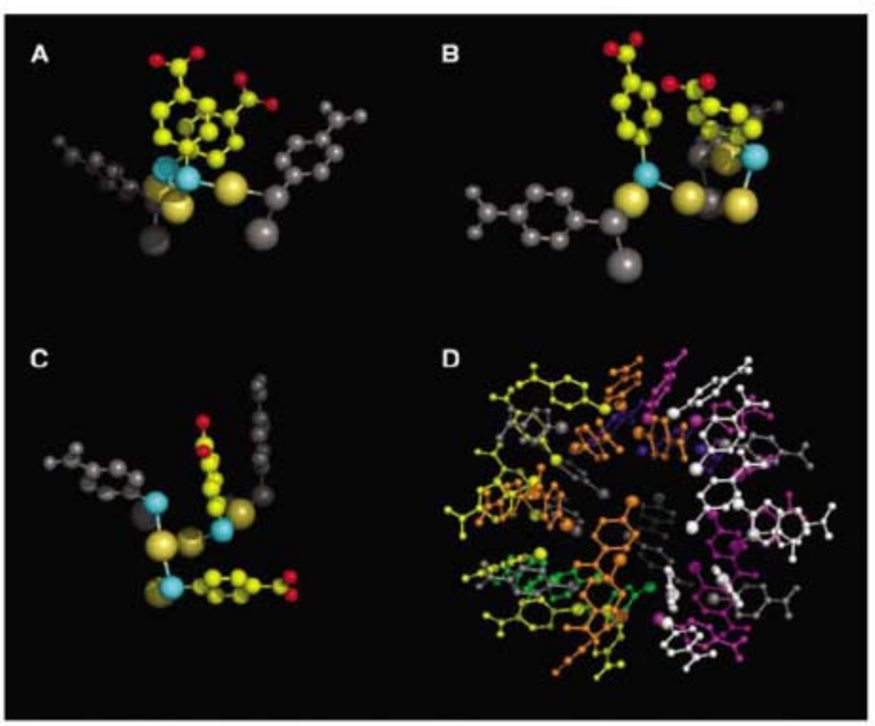


Fig. 4. p-MBA/p-MBA interactions in the surface of the nanoparticle. Color scheme as in Fig. 3B. **(A)** Phenyl rings stacked with faces parallel. **(B)** Phenyl rings stacked edge-to-face. **(C)** Phenyl ring interacting with sulfur. **(D)** Chains of interacting p-MBAs, extending across the surface of the nanoparticle, indicated by a different color for each chain.

crystals, will doubtless reveal other core structures, from which rules or principles of core assembly may ultimately be derived. It remains to investigate the chemical and physical properties of the Au_{102} nanoparticle, as well as to explore the theoretical basis of the gold packing and gold-thiol interactions that we have observed.

References and Notes

1. M. Brust, C. J. Kiely, in *Colloids and Colloid Assemblies*, F. Caruso, Ed. (Wiley-VCH, Weinheim, Germany, 2004), pp. 96–119.
2. M.-C. Daniel, D. Astruc, *Chem. Rev.* **104**, 293 (2004).
3. M. Brust *et al.*, *J. Chem. Soc. Chem. Commun.* **1995**, 1655 (1995).
4. J. P. Wilcoxon, P. P. Provencio, *J. Am. Chem. Soc.* **126**, 6402 (2004).
5. J. F. Hicks *et al.*, *Anal. Chem.* **71**, 3703 (1999).
6. R. R. Peterson, D. E. Cliffel, *Anal. Chem.* **77**, 4348 (2005).
7. T. G. Schaaff *et al.*, *J. Phys. Chem. B* **102**, 10643 (1998).
8. T. G. Schaaff, R. L. Whetten, *J. Phys. Chem. B* **103**, 9394 (1999).
9. B. L. V. Prasad *et al.*, *Langmuir* **18**, 7515 (2002).
10. M. J. Yacaman *et al.*, *J. Vac. Sci. Technol.* **19B**, 1091 (2001).
11. J. A. Ascencio *et al.*, *Surf. Sci.* **396**, 349 (1998).
12. C. L. Cleveland *et al.*, *Phys. Rev. Lett.* **79**, 1873 (1997).
13. J. D. Aiken, R. G. Finke, *J. Mol. Catal.* **145**, 1 (1999).
14. T. P. Martin, *Phys. Rep.* **273**, 199 (1996).
15. I. L. Garzon *et al.*, *Phys. Rev. B* **66**, 073403 (2002).
16. L. D. Marks, *Rep. Prog. Phys.* **57**, 603 (1994).
17. C. Vericat, M. E. Vela, R. C. Salvarezza, *Phys. Chem. Chem. Phys.* **7**, 3258 (2005).
18. M. Hasan, D. Bethell, M. Brust, *J. Am. Chem. Soc.* **124**, 1132 (2002).
19. R. S. Ingram, M. J. Hostetler, R. W. Murray, *J. Am. Chem. Soc.* **119**, 9175 (1997).
20. A. K. Boal, V. M. Rotello, *J. Am. Chem. Soc.* **122**, 734 (2000).
21. Materials and methods are available as supporting material on *Science* Online.
22. There is a slight difference between an MD and five twinned fcc or hcp crystallites, which is not discernible at the resolution of our analysis.
23. R. Bau, *J. Am. Chem. Soc.* **120**, 9380 (1998).
24. L. J. Wan *et al.*, *J. Phys. Chem. B* **104**, 3563 (2000).
25. G. Duan, V. H. Smith Jr., D. F. Weaver, *Mol. Phys.* **99**, 1689 (2001).
26. A. Ulman, *Chem. Rev.* **96**, 1533 (1996).
27. S. Wang, H. Yao, S. Sato, K. Kimura, *J. Am. Chem. Soc.* **126**, 7438 (2004).
28. R. L. Whetten *et al.*, *Acc. Chem. Res.* **32**, 397 (1999).
29. M. J. Hostetler *et al.*, *Langmuir* **14**, 17 (1998).
30. A. Herlert *et al.*, *J. Electron Spectrosc. Relat. Phenom.* **106**, 179 (2000).
31. W. A. de Heer, *Rev. Mod. Phys.* **65**, 611 (1993).
32. T. P. Martin *et al.*, *J. Phys. Chem.* **95**, 6421 (1991).
33. E. G. Mednikov, M. C. Jewell, L. F. Dahl, *J. Am. Chem. Soc.* **129**, 11619 (2007).
34. Y. Shichibu, Y. Negishi, *J. Phys. Chem. C* **111**, 7845 (2007).
35. B. K. Teo, H. Zhang, *J. Cluster Sci.* **12**, 349 (2001).
36. B. K. Teo, X. Shi, H. Zhang, *J. Cluster Sci.* **4**, 471 (1993).
37. H. Hakkinen, M. Walter, H. Gronbeck, *J. Phys. Chem. B* **110**, 9927 (2006).
38. A. C. Templeton, W. P. Wuelfing, R. W. Murray, *Acc. Chem. Res.* **33**, 27 (2000).
39. T. G. Schaaff, R. L. Whetten, *J. Phys. Chem. B* **104**, 2630 (2000).

40. We thank R. Whetten for pointing out the possibility of 58-electron shell closing and for other suggestions, G. Sheldrick for help with SHELXL and XPREP, E. Lobkovski for advice on data refinement, and H. Hakkinen, H. McConnell, and C. Chidsey for discussion and for comments on the paper. This work was supported by NSF grant CHE-0617050 and NIH grant AI21144. Portions of this research were carried out at the Stanford Synchrotron Radiation Laboratory (SSRL), a national user facility operated by Stanford University, on behalf of the U.S. Department of Energy (DOE), Office of Basic Energy Sciences. The SSRL Structural Molecular Biology Program is supported by DOE, Office of Biological and Environmental Research, and by NIH, National Center for Research Resources, Biomedical Technology Program, and the National Institute of General Medical Sciences. Portions of this research were conducted at the Advanced Light Source, a national user facility operated by Lawrence Berkeley National Laboratory, on behalf of the DOE, Office of Basic Energy Sciences. The Berkeley Center for Structural Biology is supported in part by DOE, Office of Biological and Environmental Research, and by NIH and the National Institute of General Medical Sciences. GM/CA CAT at the Advanced Photon Source has been funded in whole or in part with federal funds from the National Cancer Institute (Y1-CO-1020) and the National Institute of General Medical Sciences (Y1-GM-1104). Use of the Advanced Photon Source was supported by DOE, Office of Basic Energy Sciences, under contract no. DE-AC02-06CH11357.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/430/DC1
Materials and Methods
Figs. S1 to S10
Table S1
Structure Parameter Files

1 August 2007; accepted 13 September 2007
10.1126/science.1148624

Constraints on Neon and Argon Isotopic Fractionation in Solar Wind

Alex Meshik,[1]* Jennifer Mabry,[1] Charles Hohenberg,[1] Yves Marrocchi,[1] Olga Pravdivtseva,[1] Donald Burnett,[2] Chad Olinger,[3] Roger Wiens,[4] Dan Reisenfeld,[5] Judith Allton,[6] Karen McNamara,[6] Eileen Stansbery,[7] Amy J. G. Jurewicz[8]

To evaluate the isotopic composition of the solar nebula from which the planets formed, the relation between isotopes measured in the solar wind and on the Sun's surface needs to be known. The Genesis Discovery mission returned independent samples of three types of solar wind produced by different solar processes that provide a check on possible isotopic variations, or fractionation, between the solar-wind and solar-surface material. At a high level of precision, we observed no significant inter-regime differences in $^{20}Ne/^{22}Ne$ or $^{36}Ar/^{38}Ar$ values. For $^{20}Ne/^{22}Ne$, the difference between low- and high-speed wind components is 0.24 ± 0.37%; for $^{36}Ar/^{38}Ar$, it is 0.11 ± 0.26%. Our measured $^{36}Ar/^{38}Ar$ ratio in the solar wind of 5.501 ± 0.005 is 3.42 ± 0.09% higher than that of the terrestrial atmosphere, which may reflect atmospheric losses early in Earth's history.

Planetary materials formed from a disk of gas and dust around the early Sun, which we refer to as the solar nebula. As a standard model, planetary scientists assume that the elemental abundances and especially the isotopic compositions of elements in the nebula are uniform and that the nebular composition is preserved in the solar outer convective zone (*1*). Thus, allowing for relatively well-understood nuclear and physical/chemical isotope fractionation, terrestrial isotopic compositions should be the same as in other solar-system materials. To very high precision, this appears to be true for nonvolatile elements (*1*). However, the standard model fails for the isotopes of O, H, N, and the noble gases where large variations (compared to nonvolatile elements) are observed among terrestrial, lunar, meteoritic (asteroidal), and martian materials (*2*–*4*). Because of the nuclear conversion of D to ^{3}He, solar H is monoisotopic, and $^3He/^4He$ is greatly enhanced. Despite these exceptions, the surface layers of the Sun should preserve the nebular isotopic compositions of C, N, O, and the noble gases (*5*, *6*).

Plasma flowing from the Sun as the solar wind permits the sampling of solar matter. The Apollo Solar Wind Composition (SWC) experiment (*7*) measured relatively precise He, Ne, and Ar isotopic compositions for 1–to–3-day periods in 1969–1972. Here, we address whether the isotopic compositions of Ne and Ar, measured in the solar wind, have changed ("fractionated") from those measured on the surface of the Sun. Ulysses and Advanced Composition Explorer (ACE) spacecraft data have shown that relative proportions of elements in the solar wind are fractionated by amounts correlated with the elemental first ionization potential (FIP) (*8*). FIP fractionation presumably arises because of the preferential extraction of ions relative to atoms during transport into the solar corona from lower levels (*9*). Although the FIP is an atomic property, FIP fractionation models (*9*) predict some isotope effects, but in many specific models, these effects are small. The acceleration of heavier elements from the solar corona into the solar wind can be due to their collisions with protons ("coulomb drag"), and if the drag is incomplete,

[1]Physics Department, Washington University, St. Louis, MO 63130, USA. [2]Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA. [3]Neutron Science and Technology, MS H803, Los Alamos National Laboratory, Los Alamos, NM 87545, USA. [4]Space Science and Applications, MS D466, Los Alamos National Laboratory, Los Alamos, NM 87545, USA. [5]Physics and Astronomy Department, University of Montana, SC 121, 32 Campus Drive, MS 1080, Missoula, MT 59812, USA. [6]NASA/Johnson Space Center (JSC), Mail Code KT, Houston, TX 77058, USA. [7]NASA/JSC, Mail Code KA, Houston, TX 77058, USA. [8]Center for Meteorite Studies, Arizona State University, Mail Code 1404, Tempe, AZ 85287, USA.

*To whom correspondence should be addressed. E-mail: am@physics.wustl.edu

heavy-element density enhancements result (*10*, *11*). These enhancements depend on ion mass as well as charge and would fractionate isotopes.

Genesis exposed materials to the solar wind for 27 months (December 2001 to April 2004) (*12*). Separate samples of the three types ("regimes") of solar wind were collected. Regime-specific collector arrays were deployed according to the flow regime that was present, as determined by on-board solar-wind monitors (*13*). These regimes were: (i) low-speed, mostly less than 475 km/s but allowing for hysteresis during transitions (*13*); (ii) high-speed, onset generally 525 km/s; and (iii) coronal mass ejection (CME). CMEs are spectacular bubbles of plasma erupted in discrete events. We use the shorthand labels (i) L, (ii) H, and (iii) CME for these regimes, in addition to bulk solar wind. The quality of the regime separation has been verified by comparison with data from other spacecraft for the Genesis collection period; for instance, the H regime sample is verified to be composed of coronal-hole material by association with a low O^{7+}/O^{6+} ratio using O charge-state data from the ACE/Solar Wind Ion Composition Spectrometer instrument (*14*).

Genesis carried different collectors that were optimized for specific elements. Here, we report Ne and Ar results from aluminum-deposited-on-sapphire (AloS) collectors. The low atomic number of Al minimizes backscatter. AloS has low blanks and high noble-gas retention (*15*, *16*). AloS has probably suffered some He loss; therefore, we report only Ne and Ar results.

An infrared (IR)-laser–extraction technique was specifically designed (*17*), as it selectively evaporates only the Al film on the IR-transparent sapphire substrate. Because the laser interacts with only Al and does not impinge upon the sample-system walls, laser extraction of AloS has low blanks. Further experimental details are available in the supporting online material (*16*).

When compared with data from the Apollo SWC foils (*7*), our findings for Ne and Ar isotopic and elemental ratios validate these results but are significantly more precise (Table 1, fig. S1, and tables S1 and S2). Small (1%) but statistically significant interlaboratory variations exist between reported bulk Genesis $^{20}Ne/^{22}Ne$ values (*18*, *19*), but for assessing inter-regime differences, the precision of the data from one laboratory and instrument is what is important. To a high degree of precision, our data show no measurable differences in the $^{20}Ne/^{22}Ne$ ratio among different regime samples.

^{40}Ar is rare in solar composition but dominates atmospheric Ar because of the strong depletion of noble gases in the formation of Earth and the subsequent production from ^{40}K decay in terrestrial rocks. The measured ^{40}Ar composition (table S2) can be accounted for by system blanks with no clear contribution from particulate contamination resulting from the crash of the recovery capsule. At the 2σ level, there are no variations in $^{36}Ar/^{38}Ar$ values among the different solar-wind regime samples (Table 1). CME $^{36}Ar/^{38}Ar$ is slightly heavier than the others at 1σ but the uncertainty for this regime is almost twice as large, suggesting statistical variations. The difference between the L and H regimes for $^{36}Ar/^{38}Ar$ is 0.11 ± 0.26% (less than 1σ). Our bulk samples define a precise solar-wind $^{36}Ar/^{38}Ar$ composition of 5.501 ± 0.005.

The highest scientific objectives for Genesis are to measure the solar isotopic compositions of N and O, for which there are unexplained variations among planetary materials. Even small isotopic fractionations between the solar wind and the solar surface are of major importance for planetary sciences. Genesis independently sampled three regimes formed by different solar processes. If isotopic fractionation occurs, it is plausible that the amounts would vary among the regimes; thus the comparison of regime Ne and Ar isotopic compositions will test for fractionations. Specifically, coulomb drag effects (*11*) predict that fractionations depend directly on mass and indirectly on speed; consequently, a simple, measured quantity to assess solar-wind isotopic fractionation is the fractional difference between an L and an H regime sample. Qualitatively, light-isotope enrichment (for example, enrichment in $^{20}Ne/^{22}Ne$ values from L regime samples, relative to those from H regime samples) is predicted. The fractionation should be larger for $^{20}Ne/^{22}Ne$ than for $^{36}Ar/^{38}Ar$ because of a larger relative-mass difference. The solar He/H ratio is double that of the solar wind (*20*). If coulomb drag is a major cause of the He/H difference, then solar/solar-wind fractionation may be significantly greater than the differences between regimes.

We found no significant variations in the isotopic compositions of Ne and Ar at the 1σ level. For $^{20}Ne/^{22}Ne$, the L versus H regime difference is 0.24 ± 0.37%, corresponding to an upper limit of 0.98% at the 2σ level. Qualitatively similar limits would apply for O and N. For $^{36}Ar/^{38}Ar$, the L versus H regime difference is 0.11 ± 0.26%, corresponding to a 2σ upper limit of 0.63%.

Unlike isotopes, elemental ratios among the different regimes are not expected to be constant. Because of systematic calibration uncertainties, only relative $^{20}Ne/^{36}Ar$ results are interpretable. The $^{20}Ne/^{36}Ar$ errors (precision) (Table 1) are estimated to be about 9%, on the basis of replicate analyses of the ^{20}Ne and ^{36}Ar fluxes in the bulk sample (tables S1 and S2). At the 1σ level, $^{20}Ne/^{36}Ar$ ratios for the CME and H regime samples are the same, but L regime samples differ from the other two by about 25%. Ne has a higher FIP than Ar, so even though the solar abundances of Ne and Ar are unknown, the lower $^{20}Ne/^{36}Ar$ value for L samples indicates a greater FIP fractionation for the low-speed solar wind. This agrees with spacecraft data based on O/Si ratios (*21*). The Apollo SWC $^{20}Ne/^{36}Ar$ value is closest to our L regime value, and most of the Apollo SWC collection occurred under low-speed conditions (*7*).

A standard approach to obtaining solar abundances is to extrapolate correlation plots of solar-wind compositional variations to some known solar value (*6*). In our case, there are no variations to extrapolate; thus, our isotopic data support the previously made association of solar-wind and solar-nebula Ne and Ar isotopic compositions (*22*).

As early as the 1960s, meteoritic data on noble-gas isotopic compositions challenged the standard model of an isotopically homogeneous solar nebula. Today these data are partially understood in terms of the large contributions to bulk meteorite noble-gas concentrations by extant presolar grains (*4*). However, presolar grains cannot account for the observed O and N isotopic variations among planetary materials (*2*, *4*) and for the relatively large amounts of isotopically distinct noble gases, variously referred to as "planetary" or "Q" (*22*).

The Apollo SWC (*7*) experiment showed that the solar-wind $^{20}Ne/^{22}Ne$ ratio was highly enriched compared to the terrestrial atmospheric ratio (Table 1). This proved that volatile-element

Table 1. Ne and Ar isotopic and elemental ratios from AloS collectors. Ne is corrected for backscattering. Errors are 1σ.

Sample, collection time	$^{20}Ne/^{22}Ne$	$^{36}Ar/^{38}Ar$	$^{20}Ne/^{36}Ar$
Bulk, 852.83 days	13.972 ± 0.025	5.501 ± 0.005	59 (5)*
High-speed, 313.01 days	13.956 ± 0.041	5.502 ± 0.010	66 (6)*
CME, 193.25 days	13.979 ± 0.031	5.467 ± 0.017	59 (5)*
Low-speed, 333.67 days	13.990 ± 0.031	5.508 ± 0.010	46 (4)*
Apollo SWC (*7*)	13.7 ± 0.15	5.4 ± 0.15	49 ± 7
Terrestrial atmosphere (*28*)	9.80 ± 0.08	5.319 ± 0.011	0.524 ± 0.002
Q (*22*)	13.7	5.33	0.049

*Numbers in parentheses indicate 9% estimated errors based on the scatter in measured amounts of ^{20}Ne and ^{36}Ar.

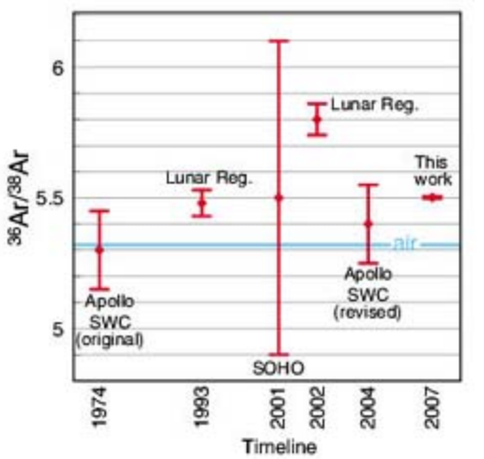


Fig. 1. Comparison of solar-wind Ar measurements: Apollo SWC foils (*7*, *28*), lunar regolith (Reg.) (*26*, *27*), SOHO (*25*), and terrestrial atmosphere (*29*).

isotopic compositions varied among planetary materials. For $^{20}Ne/^{22}Ne$, the 38% difference may reflect isotopic fractionation accompanying an early loss of the terrestrial atmosphere (*23*), an extreme example of chemical/physical mass-dependent isotope fractionation. However, the report by Clayton in 1972 of "non–mass-dependent" O isotope fractionations (*24*) clearly showed that isotopic variations in volatile elements reflect isotopic inhomogeneities in the solar nebula, an unequivocal failure of the standard model.

A major problem that remains is that of understanding the differences between solar-wind isotopic noble-gas abundances and those of meteoritic (Q) noble gases (*22*) (Table 1). These abundances should have been similar if, as expected, Q noble gases are samples of the solar nebula trapped in meteoritic material. Can a single mass-dependent fractionation process simultaneously explain large differences in $^{20}Ne/^{22}Ne$ and small but significant differences in other noble-gas isotope ratios, such as $^{36}Ar/^{38}Ar$ between Q and solar-wind noble gases? Although fractionation during adsorption onto grain surfaces has often been discussed, the fractionation process is basically unknown.

Unlike those of Ne, Ar isotopic variations, although widely believed to exist, were not quantitatively well-defined. Isotopic ratios from spacecraft instruments such as the International Solar and Heliospheric Observatory (SOHO)/mass time-of-flight spectrometer (*25*) (Fig. 1) have insufficient precision to address planetary science issues (e.g., differences between solar-wind and terrestrial $^{36}Ar/^{38}Ar$ ratios). The short Apollo SWC exposure did not yield a sufficiently precise $^{36}Ar/^{38}Ar$ value to distinguish the solar and atmospheric ratios (*7*). A higher-than-terrestrial solar $^{36}Ar/^{38}Ar$ ratio is safely inferred from studies of lunar soils, but there has been debate on how much higher it can be, with $^{36}Ar/^{38}Ar$ values reported between 5.48 and 5.80 (*26*, *27*). Corrections for excess ^{38}Ar from galactic–cosmic-ray nuclear reactions present a challenge in interpreting lunar-soil data. Genesis data require negligible galactic–cosmic-ray corrections. This, plus higher analytical precision, significantly improves the accuracy of the solar-wind and the atmospheric $^{36}Ar/^{38}Ar$ difference. Our solar-wind $^{36}Ar/^{38}Ar$ ratio (Table 1) is higher than that of the terrestrial atmosphere by 3.42 ± 0.09%. This should lead to improved constraints on models for terrestrial atmospheric loss. It is also significantly higher than the $^{36}Ar/^{38}Ar$ value of "Q".

The significantly increased precision of Ne and Ar solar-wind isotopic compositions have yielded no differences among the Genesis regime samples. Nevertheless, we have not proved that there is no Ne or Ar isotopic fractionation between the Sun and the solar wind. Our data clearly put a constraint on such fractionations, but at present the strength of the constraint is not known. A "unified" theoretical approach evaluating FIP and coulomb drag fractionations in the context of formation models of the different solar-wind regimes is required. If fractionations between the Sun and the solar wind are large but the same for all regimes, then the absence of inter-regime isotopic variations is inconclusive. Alternatively, the fractionations among regimes could be large and the lack of inter-regime variations quite constraining. The goal of the Genesis mission is to provide higher-precision solar-wind composition data, leading to better theories of solar-wind fractionations, which, in turn, will lead to improved solar abundances for planetary science purposes.

References and Notes

1. H. Palme, A. Jones, in vol. 1 of *Treatise of Geochemistry*, A. M. Davis, Ed. (Elsevier, Amsterdam, 2003), chap. 3, pp. 41–61.
2. R. N. Clayton, in vol. 1 of *Treatise of Geochemistry*, A. M. Davis, Ed. (Elsevier, Amsterdam, 2003), chap. 6., pp. 129–142.
3. H. Busemann *et al.*, *Science* **312**, 727 (2006).
4. R. Wieler, H. Busemann, I. Franchi, in *Meteorites and the Early Solar System II*, D. S. Lauretta, H. McSween Jr., Eds. (Univ. of Arizona, Tucson, AZ, 2006), part vi, pp. 499–521.
5. Nuclear-energy generation in the interior of the Sun has produced significant elemental and isotopic changes, but matter is not exchanged between the inner and outer regions, so with a few exceptions, the composition of the solar surface has remained unchanged since the beginning of the solar system.
6. J. Geiss, G. Gloeckler, *Space Sci. Rev.* **106**, 3 (2003).
7. J. Geiss *et al.*, *Space Sci. Rev.* **110**, 307 (2004).
8. R. von Steiger *et al.*, *Space Sci. Rev.* **97**, 123 (2001).
9. R. von Steiger, J. Geiss, *Astron. Astrophys.* **225**, 222 (1989).
10. J. Geiss, P. Hirt, H. Leutwyler, *Solar Phys.* **12**, 458 (1970).
11. R. Bodmer, P. Bochsler, *Astron. Astrophys.* **337**, 921 (1998).
12. D. S. Burnett *et al.*, *Space Sci. Rev.* **105**, 509 (2003).
13. M. Neugebauer *et al.*, *Space Sci. Rev.* **105**, 661 (2003).
14. D. B. Reisenfeld *et al.*, *Am. Inst. Phys. Conf. Proc.* **679**, 632 (2003).
15. J. C. Mabry *et al.*, *Lunar Planet. Sci. Conf.* **XXXVIII**, abstr. 1338 (2007).
16. Materials and methods are available as supporting material on *Science* Online.
17. C. M. Hohenberg, N. Thonnard, A. Meshik, *Meteorit. Planet. Sci.* **37**, 257 (2002).
18. V. S. Heber, H. Baur, D. Burnett, R. Wieler, *Lunar Planet. Sci. Conf.* **XXXVIII**, abstr. 1894 (2007).
19. A. Grimberg *et al.*, *Science* **314**, 1133 (2006).
20. S. Basu, H. M. Antia, *Astrophys. J.* **606**, L85 (2004).
21. J. Geiss, G. Gloeckler, R. von Steiger, *Space Sci. Rev.* **72**, 49 (1995).
22. M. Ozima, R. Wieler, B. Marty, F. Podosek, *Geochim. Cosmochim. Acta* **62**, 301 (1998).
23. R. O. Pepin, *Earth Planet. Sci. Lett.* **252**, 1 (2006).
24. R. N. Clayton, *Earth Planet. Sci. Lett.* **13**, 455 (1972).
25. J. M. Weygand, F. Ipavich, P. Wurz, J. Paquette, P. Bochsler, *Geochim. Cosmochim. Acta* **65**, 4589 (2001).
26. J.-P. Benkert, H. Baur, P. Signer, R. Wieler, *J. Geophys. Res. (Planets)* **98**, 13147 (1993).
27. R. L. Palma, R. Becker, R. Pepin, D. Schlutter, *Geochim. Cosmochim. Acta* **66**, 2929 (2002).
28. H. Cerutti, thesis, Univ. of Bern (1974).
29. A. O. Nier, *Phys. Rev.* **77**, 789 (1950).

30. We acknowledge the support of the entire Genesis team in enabling this work. Portions of this work at Washington Univ. were supported by NASA grants (NNJ04HI17G and NAG5-12885).

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/433/DC1
SOM Text
Fig. S1
Tables S1 and S2
References

22 May 2007; accepted 31 August 2007
10.1126/science.1145528

Southern Hemisphere and Deep-Sea Warming Led Deglacial Atmospheric CO_2 Rise and Tropical Warming

Lowell Stott,[1]* Axel Timmermann,[2] Robert Thunell[3]

Establishing what caused Earth's largest climatic changes in the past requires a precise knowledge of both the forcing and the regional responses. We determined the chronology of high- and low-latitude climate change at the last glacial termination by radiocarbon dating benthic and planktonic foraminiferal stable isotope and magnesium/calcium records from a marine core collected in the western tropical Pacific. Deep-sea temperatures warmed by ~2°C between 19 and 17 thousand years before the present (ky B.P.), leading the rise in atmospheric CO_2 and tropical–surface-ocean warming by ~1000 years. The cause of this deglacial deep-water warming does not lie within the tropics, nor can its early onset between 19 and 17 ky B.P. be attributed to CO_2 forcing. Increasing austral-spring insolation combined with sea-ice albedo feedbacks appear to be the key factors responsible for this warming.

The data obtained from high-latitude ice cores establish a close temporal relation between varying concentrations of atmospheric CO_2 and atmospheric temperatures during glacial terminations (*1*). However, uncertainty in the gas-age chronologies and inadequate temporal resolution in many proxy climate reconstructions have hampered efforts to establish the exact phasing of events during glacial terminations, a necessary step in understanding the physical relation between CO_2 forcing and climate change (*2*). Arguably, the most robust estimate of changes in mean global temperatures that accompany glacial terminations is the amount of heat stored in the oceans. The large size of the oceanic reservoir and the long residence time of

deep waters mean that deep-water temperatures reflect a globally averaged record of Earth's radiative-heat balance (*3*). At present, we cannot quantify changes in ocean heat content at the last glacial termination. However, because deep waters formed at high latitudes carry with them the nearly conservative properties of temperature and salinity, it is possible to establish the relative timing of high-latitude versus low-latitude climatic change at glacial terminations by dating co-occurring benthic (bottom-dwelling) and planktonic (surface-dwelling) foraminiferal paleotemperature records in a marine core from a tropical location that has sufficient temporal resolution. We used radiocarbon (^{14}C) dating to establish the timing of the deep-sea and tropical–surface-ocean temperature changes during the last glacial termination and compared this history with the timing of CO_2 change and deglacial warming in the southern high latitudes during the last glacial termination.

Marine sediment core MD98-2181 from the Morotai Basin (Fig. 1) is ideally suited for documenting the timing of both deep-sea and tropical–surface-water temperature change during the last glacial termination because the sediments at this site are an admixture of planktonic and benthic foraminiferal carbonate that accumulated at rates of 50 to 80 cm/thousand years (ky) over the past glacial-to-interglacial transition (*4*). Sampling this core at the centimeter scale provides a temporal resolution for each sample of ~25 to 50 years (*5*). This core was recovered from a depth of 2114 m, where it was bathed in the upper Pacific Deep Water, a water mass whose temperature and salinity are acquired within the Southern Ocean (*6*). The site is also located within the Pacific Warm Pool (*7*), where the $\delta^{18}O$ and Mg/Ca of planktonic foraminifera record the temperature and salinity of western tropical Pacific surface water (*8*, *9*).

We compiled 42 planktonic (*4*) and 8 mixed-benthic (Table 1) ^{14}C ages from late glacial and Holocene sections of this site (Fig. 2). Broecker *et al.* (*10*) noted previously that the benthic/planktonic ^{14}C age difference from the glacial termination horizons of this core averages ~1300 years, similar to the ^{14}C offset between surface and deep waters today (fig. S2). Of the eight benthic/planktonic pairs we have from the deglacial section, the average age difference is 1493 (±367) years, similar to Broecker *et al.*'s estimate. The reservoir age of surface waters in the source region of the Pacific Deep Water may have been slightly older during the glacial period, which would explain the somewhat higher surface/deep-water age difference in the glacial samples (*11*). However, given the small range in $\Delta^{14}C$, we

[1]Department of Earth Sciences, University of Southern California, Los Angeles, CA 90089, USA. [2]International Pacific Research Center (IPRC), School of Ocean and Earth Science and Technology, University of Hawaii, Honolulu, HI 96822, USA. [3]Department of Geological Sciences, University of South Carolina, Columbia, SC 29208, USA.

*To whom correspondence should be addressed. E-mail: stott@usc.edu

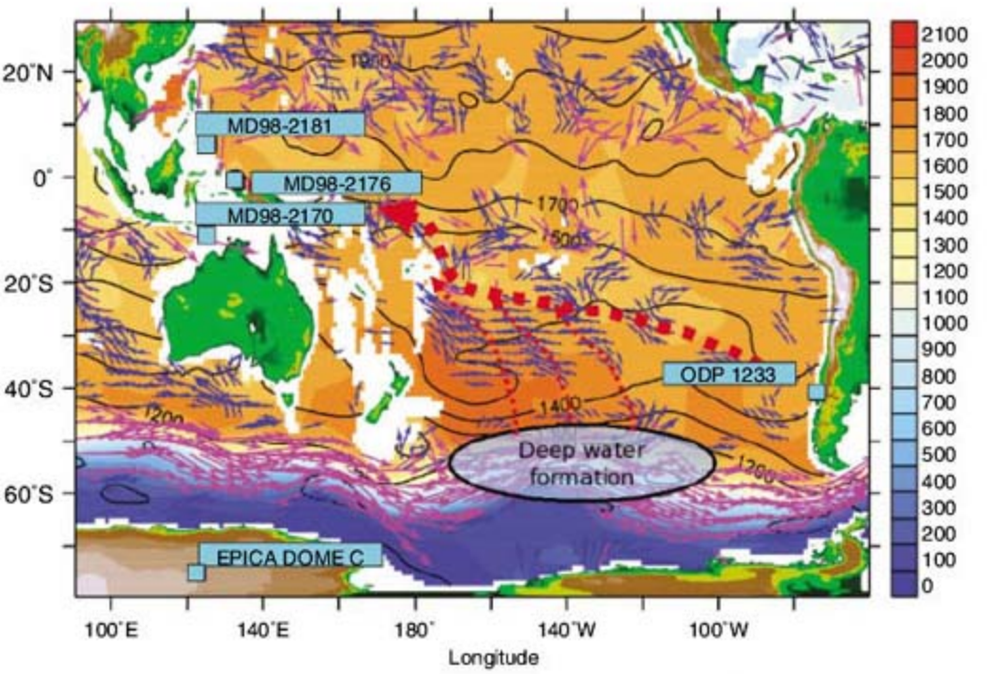


Fig. 1. Location of tropical sites (MD98-2181, MD98-2176 and MD98-2170), high–southern latitude Ocean Drilling Program site 1233, and the EPICA Dome C ice core. At 2100 m, the MD98-2181 site is bathed by the Pacific Deep Water (supporting online material). The depth of the isopycnal surface σ_t = 27.6 kg/m^3 (relative to the 0 m) is shown with color shading (*39*), pre-bomb ^{14}C ages from the Global Data Analysis Project data set (*40*) are interpolated onto σ_t = 27.6 kg/m^3 (contour lines), geostrophic velocity on σ_t = 27.6 kg/m^3 is shown (blue and magenta vectors, the scale of the magenta vectors is reduced by 60% relative to the blue vectors for better visibility), and the locations of marine cores are indicated.

Table 1. Benthic foraminiferal ^{14}C ages from the Morotai Basin core MD98-2181 (6°N, 126°E, 2.1 km). All ^{14}C measurements were conducted at the Woods Hole Accelerator Laboratory. NOSAMS, National Ocean Sciences Accelerator Mass Spectrometry.

Depth (cm)	NOSAMS accession number	Benthic species	^{14}C age (years)	Error (years)
751	OS-55868	*C. mundula*	8,950	50
861	OS-55869	*C. mundula*	10,350	65
1086	OS-54924	*C. mundula*	14,550	70
1211	OS-54928	Mixed benthics	17,150	85
1286	OS-54925	Mixed benthics	18,500	130
1486	OS-55820	Mixed benthics	21,800	95
1561	OS-60238	Mixed benthics	23,600	160
1711	OS-60246	Mixed benthics	26,900	120

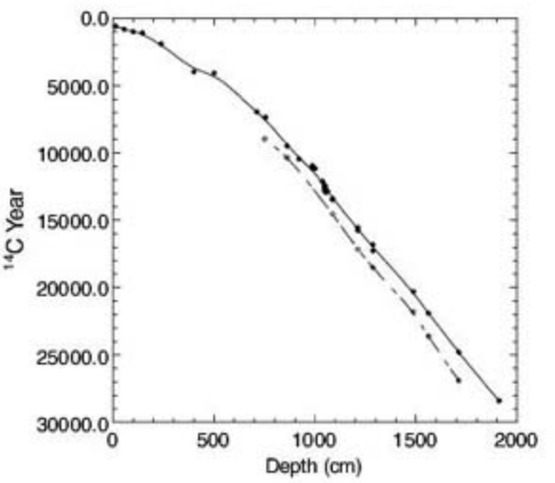


Fig. 2. ^{14}C ages of planktonic (solid circles) and benthic foraminifers (open circles) from MD98-2181. The average benthic/planktonic ^{14}C age difference is 1493 (±367) years through the late glacial and early deglacial portion of the core. ^{14}C dates for terrestrial wood from the glacial section indicates that the surface reservoir age in the Western tropical Pacific was not much higher during the glacial period (*10*). ^{14}C data from 941 to 961 cm were excluded because of a sediment disturbance at this depth (*4*).

see no evidence for a substantial shift in the surface/deep-water age difference and therefore estimate that the transit time of deep water from the Southern Ocean to the north equatorial Pacific was ~1000 (±300) years during the glacial period, as in the modern ocean (*12*). The conversion of ^{14}C ages to a calendar-age model was performed with the CALIB 5.0.2 routine using the Marine04 calibration data set (*13*). A reservoir-age correction of 560 years was applied for the planktonic samples younger than 13,000 years; a correction of 630 years was applied for older samples. At each stratigraphic horizon, the benthic foraminiferal $\delta^{18}O$ value reflects the deep-water temperature and oxygen isotope composition acquired from the Southern Ocean ~1000 years earlier (fig. S2). Therefore to compare the temporal phasing of deep-water– and tropical–surface-water changes at the deglaciation, an independent chronology was established for the benthic $\delta^{18}O$ record by adding 1000 years to the planktonic calendar age at each core horizon.

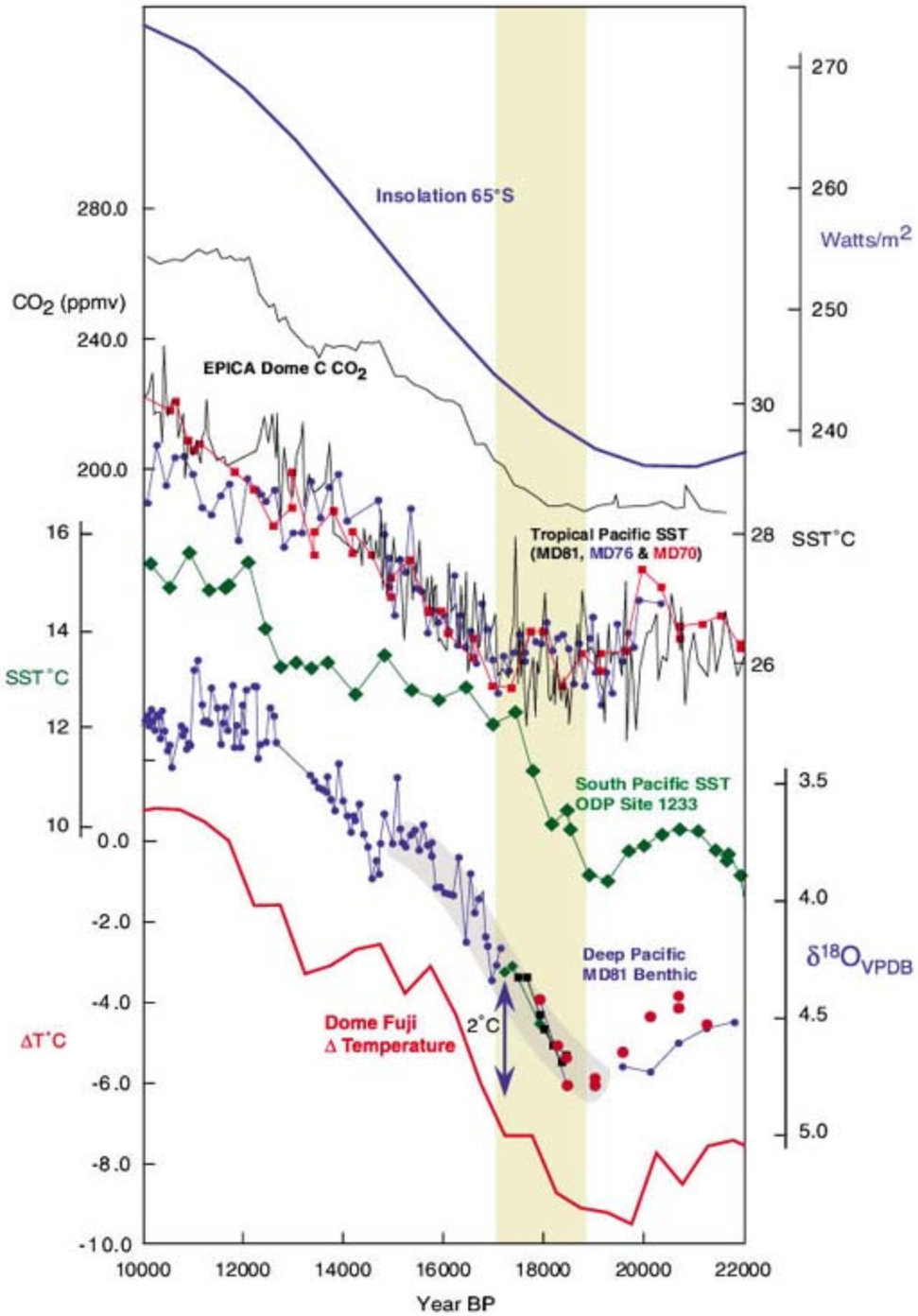


Fig. 3. Temporal phasing of the Pacific deep- and tropical–surface-water deglacial stratigraphy compared with the EPICA Dome C CO_2 record (*1*). The averaged mean longitude spring insolation (21 August to 20 November) at 65°S insolation and the Dome Fuji ice core temperature deviation (ΔT_{site}) relative to the mean of the last 10 thousand years is shown (*34*). The yellow shading spans the time period between the initial warming of deep-sea temperatures and the onset of tropical Pacific SST warming. The Dome C CO_2 record was plotted on the GISPII gas-age model (*15*) by synchronizing the GISP II and Dome C CH_4 stratigraphies. The deep Pacific $\delta^{18}O$ record is based on multiple species of benthic foraminifera from MD98-2181 (*Cibicidoides mundula*, blue circles; *Uvigerina costata*, red circles; *Uvigerina hispida*, black squares; *Cibicidoides wuellerstorfii*, green diamonds; red squares, *Uvigerina* sp.) and is depicted on an age scale that represents the surface conditions at the source region of the Pacific Deep Water. The gray shading indicates a ~200-year uncertainty in the benthic ages. A disturbed interval between the 941- and 961-cm sections of MD98-2181 encompassing the interval associated with the Antarctic Cold Reversal is excluded. ppmv, parts per million by volume; VPDB, Vienna Pee Dee belemnite.

Previous studies (*8*, *9*, *14*–*18*) have shown that during the last glacial termination, tropical Pacific sea surface temperatures (SSTs) began to warm at ~17.5 thousand years before the present (ky B.P.) and that the warming preceded the first major shift in seawater $\delta^{18}O$ associated with the disintegrating glacial ice sheets (*17*). The timing of tropical SST warming is best documented in the MD98-2181 core, which has the highest sediment-accumulation rate and the most detailed ^{14}C age control of the available cores (fig. S1). However, two other closely associated cores, MD98-2176 and MD98-2170, compliment the MD98-2181 SST record and provide a composite representation of the Western Pacific Warm Pool during the last deglaciation (*8*). During the Last Glacial Maximum, the SSTs at these sites averaged 26°C (Fig. 3) (*8*, *9*). SSTs increased by ~1°C between 17 and 16 ky B.P., and by 14.6 ky B.P. they had reached ~28°C across the Warm Pool. The deglacial warming within the tropical Pacific Warm Pool occurred in close association with increasing concentrations of atmospheric CO_2 (*14*); this finding has provided support for hypotheses that call on the CO_2 increase itself to explain the deglacial warming (*19*–*21*). However, the exact phasing between the CO_2 increase and the tropical SST warming is not well established because of the uncertainty in assigning gas ages to CO_2 records (*1*, *22*–*24*). In the Dome C ice core, for example, the concentrations of both CO_2 and CH_4 begin to increase above their glacial values at 525 m. In the European Project for Ice Coring in Antarctica (EPICA) Dome C 2 (*1*) time scale, this corresponds to a gas age of 17.3 ky B.P., which would mean that the initial rise in CO_2 occurred essentially simultaneously with warming in the tropical Pacific. However, by synchronizing CH_4 records from the Antarctic Dome C core and the Greenland Ice Sheet Project (GISP) core (*23*) and adopting the GISPII gas-age model, the initial CO_2 rise could be shifted to as early as 18 ky B.P. and therefore slightly earlier than the tropical SST warming (Fig. 3). In either case, the concentration of atmospheric CO_2 did not begin to rise until after 19 ky B.P.

If atmospheric CO_2 was the sole driver of deglacial temperature changes, the deep-sea temperatures should reflect this with an appropriate lagged relation that would account for the response time between CO_2 forcing and the turnover time of deep waters. The Pacific is the best candidate for testing the temporal relation because

it is volumetrically the largest deep-water reservoir. Shackleton (*19*) recognized the phasing similarity between $\delta^{18}O$ of O_2 gas trapped in the Vostok ice core (*25*) and the orbital-insolation record (June insolation at 65°N) and argued that the O_2 record reflects the influence of solar-insolation forcing vis-à-vis ice-volume variations. He tuned the deep-sea (benthic) $\delta^{18}O$ record from Pacific core VM19-30 to the Vostok O_2 $\delta^{18}O$ stratigraphy and applied the orbital-insolation chronology to it. The advantage of this approach would be to provide an estimate of the deep-sea temperature change, which is the residual benthic $\delta^{18}O$ signal that is not accounted for by the Vostok O_2 $\delta^{18}O$ record. However, this method also imposed a phase relation between the deep-sea temperature change and the ice-volume components of the benthic $\delta^{18}O$ record. As a result, the "tuned" VM19-30 benthic $\delta^{18}O$ record does not exhibit any deglacial $\delta^{18}O$ change until ~15 ky B.P., nearly 3000 years after atmospheric CO_2 began to rise (fig. S2). In a subsequent study of deep Pacific temperature changes during the last glacial termination, Martin *et al.* (*20*) used Mg/Ca–based estimates of benthic temperature change to argue that deep Pacific temperatures warmed by 2°C during the glacial termination and that the magnitude of these deep-sea temperature changes was consistent with a forcing by CO_2 during the deglaciation. Here again, the issue is timing. Did deep-sea temperature change lag the CO_2 forcing? The records used by Martin *et al.* (*20*) to argue for the CO_2 forcing of deep-sea temperatures were also tuned to the orbital-insolation record through a cross correlation of benthic $\delta^{18}O$ records to Shackleton's VM19-30 record. Consequently, these cores do not provide an independent constraint on the temporal relation between temperature change and CO_2 concentration.

We circumvented the assumptions associated with tuning benthic records by independently dating benthic foraminifera from MD98-2181 (Table 1). In Fig. 3, the independently dated MD98-2181 benthic $\delta^{18}O$ stratigraphy (representing Southern Ocean surface conditions 1000 years earlier) is compared with the western tropical Pacific SST reconstructions. The maximum benthic $\delta^{18}O$ values occurred between 20 and 19 ky B.P. Three species of benthic foraminifera exhibited a $\delta^{18}O$ value of 4.8 per mil (‰) during this time period, a value that is 1.8‰ higher than modern benthic values at this site. Taking the mean ocean glacial-to-interglacial $\delta^{18}O$ shift to be 1‰ (*19*, *26*–*30*), the observed 1.8‰ difference between Holocene and glacial $\delta^{18}O$ values at MD98-2181 implies that temperatures at 2100 m in the Pacific were ~2.5°C colder and/or the $^{18}O/^{16}O$ of the deep water was substantially higher at the glacial maximum. Pore-water Cl profiles from deep-sea sediment cores suggest that deep waters were more salty during the Last Glacial Maxmium, perhaps by as much 1‰ (*30*), but there is no evidence to suggest that the $^{18}O/^{16}O$ of the Pacific deep water was substantially higher (after correcting for the 1‰ ice-volume effect) (*31*). Considering these results together with the Mg/Ca data from the eastern Pacific (*32*) indicates that the primary influence on the early deglacial benthic $\delta^{18}O$ evolution was temperature change. The benthic $\delta^{18}O$ values from MD98-2181 began to decrease from the cold glacial maximum values at 19 to 18.5 ky B.P. and decreased progressively by ~0.5‰ over the next 1000 years. If this 0.5‰ decrease was entirely due to temperature, it means that nearly all of the glacial/interglacial deep-water warming occurred before 17.5 ky B.P. and therefore before both the onset of deglacial warming in tropical Pacific surface waters and the increase in atmospheric CO_2 concentration.

A comparable warming has also been observed in surface-water records from the mid-latitudes in the south Pacific (*33*). At this latitude the surface waters also warmed by 2°C between 19 and 17 ky B.P., leading the tropical warming by ~1000 years (Fig. 2). The onset and temporal evolution of deglacial warming over the Antarctic continent (*34*) also matches the deep-ocean record of warming (Fig. 3). These records, together with the synchronous retreat of mid–southern-latitude glaciers (*35*), confirm that the onset of deglacial warming throughout the Southern Hemisphere occurred long before deglacial warming began in the tropical surface ocean. An early warming in the Antarctic has important implications for understanding what may have caused an asynchronous deglacial warming pattern between the low and high southern latitudes. In particular, it means that the mechanism responsible for initiating the deglacial events does not lie directly within the tropics itself, nor can these events be explained by CO_2 forcing alone. Both CO_2 and the tropical SSTs did not begin to change until after 18 ky B.P. (*22*), approximately 1000 years after the benthic $\delta^{18}O$ record indicates that the Southern Ocean was warming.

The rise in Southern Ocean temperatures coincided with the retreat of Antarctic sea ice and high-elevation glaciers in the Southern Hemisphere (*36*, *37*). The explanation for the early warming in the Southern Hemisphere could involve increasing springtime solar insolation, which is well correlated with the retreat of sea ice and with the history of sea-salt accumulation in the Antarctic Dome C ice core (fig. S5). We suggest that the trigger for the initial deglacial warming around Antarctica was the change in solar insolation over the Southern Ocean during the austral spring that influenced the retreat of the sea ice (*38*). Retreating sea ice would have led to enhanced Ekman transport in the Southern Ocean and decreased stratification due to stronger air-sea fluxes. We hypothesize that these forcings promoted enhanced ventilation of the deep sea and the subsequent rise in atmospheric CO_2.

References and Notes

1. EPICA Community Members, *Nature* **429**, 623 (2004).
2. P. Köhler, H. Fischer, G. Munhoven, R. E. Zeebe, *Global Biogeochem. Cycles* **19**, GB4020 (2005).
3. T. P. Barnett, D. W. Pierce, R. Schnur, *Science* **292**, 270 (2001).
4. L. Stott, *Paleoceanography* **22**, PA1211 (2007).
5. Materials and methods are available as supporting material on *Science* Online. In the supporting material, we also present our ^{14}C data and age model details for cores MD98-2176 and MD98-2170 (fig. S1).
6. Details about the Pacific Deep Water formation and circulation are given in the supporting material.
7. M. Ioualalen *et al.*, *Geophys. Res. Lett.* **27**, 1243 (2000).
8. L. Stott *et al.*, *Nature* **431**, 56 (2004).
9. L. Stott, C. Poulsen, S. Lund, R. Thunell, *Science* **297**, 222 (2002).
10. W. Broecker *et al.*, *Science* **306**, 1169 (2004).
11. E. L. Sikes, C. R. Samson, T. P. Guilderson, W. R. Howard, *Nature* **405**, 555 (2000).
12. The distribution pre-bomb ^{14}C ages within the Pacific are illustrated in fig. S3. The ^{14}C age of the Pacific Deep Water increases by ~1000 years between the Southern Ocean and the tropical Pacific.
13. K. A. Hughen *et al.*, *Radiocarbon* **46**, 1059 (2004).
14. D. W. Lea, D. K. Pak, H. J. Spero, *Science* **289**, 1719 (2000).
15. M. K. Gagan, E. J. Hendy, S. G. Haberle, W. S. Hantoro, *Quat. Int.* **118–119**, 127 (2004).
16. D. W. Oppo, B. K. Linsley, Y. Rosenthal, S. Dannenmann, L. Beaufort, *Geochem. Geophys. Geosyst.* **4**, 1003 (2003).
17. K. Visser, R. Thunell, L. Stott, *Nature* **421**, 152 (2003).
18. T. Kiefer, M. Kienast, *Quat. Sci. Rev.* **24**, 1063 (2005).
19. N. J. Shackleton, *Science* **289**, 1897 (2000).
20. P. Martin, D. Archer, D. W. Lea, *Paleoceanography* **20**, PA2015 (2005).
21. D. W. Lea, *J. Clim.* **17**, 2170 (2004).
22. E. Monnin *et al.*, *Science* **291**, 112 (2001).
23. T. Blunier, E. J. Brook, *Science* **291**, 109 (2001).
24. L. Loulergue *et al.*, *Clim. Past Discuss.* **3**, 435 (2007).
25. J. R. Petit *et al.*, *Nature* **399**, 429 (1999).
26. J. F. Adkins, D. P. Schrag, *Geophys. Res. Lett.* **28**, 771 (2001).
27. K. Lambeck, J. Chappell, *Science* **292**, 679 (2001).
28. D. P. Schrag, G. Hampt, D. W. Murray, *Science* **272**, 1930 (1996).
29. J. Chappell, *Quat. Sci. Rev.* **21**, 1229 (2002).
30. J. F. Adkins, K. McIntyre, D. P. Schrag, *Science* **298**, 1769 (2002).
31. D. P. Schrag *et al.*, *Quat. Sci. Rev.* **21**, 331 (2002).
32. P. A. Martin *et al.*, *Earth Planet. Sci. Lett.* **198**, 193 (2002).
33. J. Kaiser, F. Lamy, D. Hebbeln, *Paleoceanography* **20**, PA4009 (2005).
34. K. Kawamura *et al.*, *Nature* **448**, 912 (2007).
35. J. M. Schaefer *et al.*, *Science* **312**, 1510 (2006).
36. A. Shemesh *et al.*, *Paleoceanography* **17**, 1056 (2002).
37. T. T. Barrows, J. O. Stone, L. K. Fifield, *Quat. Sci. Rev.* **23**, 697 (2004).
38. The sea-salt Na accumulation in Antarctic ice cores (fig. S5) is interpreted here as a proxy for sea-ice coverage around Antarctica.
39. S. Levitus, Climatological Atlas of the World Ocean (U.S. Government Printing Office, Washington, DC, 1994).
40. R. M. Key *et al.*, *Global Biogeochem. Cycles* **18**, GB4031 (2004).

41. L.S., R.T., and A.T. were supported by grants from the Marine Geology and Geophysics and the Atmospheric Sciences Paleoclimatology Programs of NSF. A.T. is supported by the Japan Agency for Marine-Earth Science and Technology through its sponsorship of the International Pacific Research Center. This is IPRC Publication number 471. We thank M. Rincon for analytical support and O. Timm for many stimulating discussions.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1143791/DC1
Materials and Methods
SOM Text
Figs. S1 to S5
Table S1
References

13 April 2007; accepted 11 September 2007
Published online 27 September 2007;
10.1126/science.1143791
Include this information when citing this paper.

Mixed-Layer Deepening During Heinrich Events: A Multi-Planktonic Foraminiferal $\delta^{18}O$ Approach

Harunur Rashid[1,2]* and Edward A. Boyle[2]

Proxies from Greenland ice cores and North Atlantic marine sediment cores document repeated extreme climate swings of a few decades to millennia during the last glacial cycle, including periods of intense ice rafting called Heinrich events (HEs). We have found similar oxygen isotope variations recorded in mixed-layer– and thermocline-dwelling planktonic foraminifera during HEs 0, 1, and 4, suggesting that three foraminiferal taxa calcified their shells at similar temperatures in a homogenized upperwater column. This implies that the surface mixed layer was deeper during HEs. Similar deepening occurred on the northern margin of the ice-rafted–debris belt, implying that these deep mixed layers during HEs were widespread in the region. We suggest that an increase in storminess during HEs intensified the vertical mixing of meltwater from ice rafting in the upper ocean.

Late Pleistocene Heinrich events (HEs) (*1*–*3*) in the North Atlantic are identified by distinct ice-rafted–detritus (IRD) layers primarily derived from the Laurentide Ice Sheet (*4*). Eight such events coincided with the coldest, stadial phase of some Dansgaard-Oeschger (D/O) events in Greenland ice cores over the past ~65,000 years. D/O events typically start with an abrupt 9 to 15°C (*5*) warming over a few decades or less, followed by gradual cooling over several millennia. The potential of HEs to reduce or halt North Atlantic Deep Water (NADW) formation by preventing convection in the northern North Atlantic and hence the northward penetration of the North Atlantic Current (NAC), with implications for high-latitude heat budgets, has been explored (*6*, *7*). Numerical modeling studies support the notion that freshwater forcing would be capable of substantially altering the site and strength of NADW formation (*6*–*8*). Most earlier studies used single species of planktonic foraminifera to document the freshening and cooling of the surface of the North Atlantic during HEs. As a result, the detailed impact of these fresh waters on upper water masses (i.e., the mixed-layer and thermocline waters) could not be assessed. We used oxygen isotopes on multiple planktonic foraminifera to show that a substantial perturbation of the entire upper water masses occurred during HEs.

HEs were identified by peaks in IRD (%), *Neogloboquadrina pachyderma* [sinistral (s)] (%), and lighter oxygen isotopes in planktonic foraminifera (*2*) [supporting online material (SOM) text, Methods] in sediments from Chain 82 Station 50 Core 20 (CHN82-20) piston and patching trigger cores (43.30°N, 29.52°W; 3020 m of water depth) (*9*). CHN82-20 was retrieved at the southern margin of the IRD belt (*10*) and thus preserves a record of ice rafting, but it was situated south of the polar front even during the Last Glacial Maximum (LGM) (Fig. 1). Oxygen isotopic analyses were performed in planktonic foraminifera *N. pachyderma* (s) and dextral (d) and *Globigerina bulloides* (SOM text).

The identification of HEs was constrained by 16 published (*9*, *11*) and new radiocarbon (^{14}C) accelerator mass spectrometry (AMS) dates and the identification of the Vedde Ash Zone (*11*) (table S1). ^{14}C AMS ages were calibrated to calendar years before the present (cal yr B.P.) (*12*, *13*). The sedimentation rate varied from 2.15 to 4.5 cm/thousand years (ky) during the Holocene. Six ^{14}C AMS dates between 35 and 44.5 cm and between 74.25 and 80 cm suggest that during the HE0 and HE1 periods, sedimentation rates could have been as high as 20 and 28 cm/ky (*11*), respectively. Mean sedimentation rates are ~5 cm/ky in marine isotope stage (MIS) 3.

HE1, HE4, and HE5 are identified by an increase in % IRD and % *N. pachyderma* (s), a decrease in the concentration of total planktonic foraminifera, and a lighter $\delta^{18}O$ in planktonic foraminifera (Fig. 2). HE0 is identified by a decrease in the concentration of foraminifera, an increase in % *N. pachyderma* (s), and a lighter $\delta^{18}O$ in planktonic foraminifera. The absence of IRD in HE0 suggests a subdued ice-rafting event restricted to the northern latitudes (*2*, *14*). HE2 is identified by a modest increase in % IRD and low foraminiferal concentration. HE3 is less well defined, but the low foraminiferal concentration, a single peak in % IRD, and a lighter $\delta^{18}O$ in *N. pachyderma* (d) mark its presence. HE4 has well-defined % IRD and % *N. pachyderma* (s) peaks at the beginning and a more subdued peak in % IRD during the latter part, as observed elsewhere in the IRD belt (*15*). HE5 exhibits two IRD and % *N. pachyderma* (s) peaks. The basal IRD peaks in H4 and H5 are dominantly composed of detrital carbonate, whereas the latter IRD peaks are of a mixture of detrital grains of various origins.

In the area of the core site, *G. bulloides* has been identified as a mixed-layer–dwelling species commonly found between 0 and ~30 m of water depth (*16*–*18*). The distribution of *G. bulloides* is associated with the spring bloom (*16*, *19*) in the North Atlantic. *N. pachyderma* (d) lives at slightly deeper depths (between ~30 and 100 m) (*19*, *20*), and *N. pachyderma* (s) is a deep-dwelling species commonly found at water depths ranging from ~100 to 250 m (i.e., in the upper thermocline) in the polar to subpolar environment. Holocene stratification of upper water masses is manifested in divergent $\delta^{18}O$ values of the different foraminiferal species (Fig. 3A). Our oxygen isotope data show that the *G. bulloides* $\delta^{18}O$ displays the lightest values, whereas *N. pachyderma* (s) consistently shows enriched values and *N. pachyderma* (d) shows intermediate values. However, converging $\delta^{18}O$ values were measured in *N. pachyderma* (s and d) and *G. bulloides* consistently in HE0, HE1, and HE4 (Fig. 3B), demonstrating that during HEs all three planktonic foraminifera calcified at similar hydrographic conditions (that is, temperature and $\delta^{18}O$ of surface water). HE0 differs slightly from the other HEs by exhibiting $\delta^{18}O$

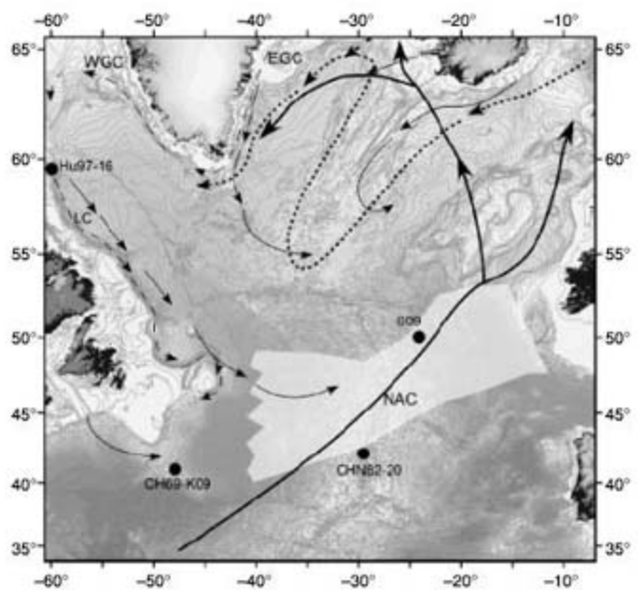


Fig. 1. Location of cores discussed in this study. The trajectory path of the icebergs (thin black arrows) and those of the NADW (thick dotted lines), Labrador Current (LC), West and East Greenland Currents (WGC and EGC) (thin dashed lines), and NAC (thick solid lines) are shown. The position of the IRD belt is shown by the gray shaded area, and iceberg routes and NAC are drawn according to Ruddiman (*10*).

[1]College of Marine Science, University of South Florida, 140 7th Avenue South, St. Petersburg, FL 33701, USA. [2]Department of Earth and Planetary Sciences, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139, USA.

*To whom correspondence should be addressed. E-mail: hrashid@marine.usf.edu

that is lighter for *N. pachyderma* (s and d) and enriched for *G. bulloides*. Conversely, during the non-Heinrich intervals, distinct $\delta^{18}O$ divergence among these planktonic foraminiferal species suggests that stratification in near-surface waters was reestablished at the core site.

The planktonic foraminifera did not respond uniformly during all HEs. During HE5, *N. pachyderma* (d) and *G. bulloides* show identical $\delta^{18}O$ values, whereas *N. pachyderma* (s) exhibits heavier values. Both *N. pachyderma* (s and d) show lighter $\delta^{18}O$ values during HE3, whereas $\delta^{18}O$ in *G. bulloides* did not change from pre-HE3 conditions. Lighter $\delta^{18}O$ is not recorded in the planktonic foraminifera during HE2. These data suggest intense mixing of upper waters during HE0, HE1, and HE4 and, by extension, suggest homogenization of the upper 250 m, despite the freshwater input from melting icebergs. The earlier IRD peak in HE4 precedes the conditions with similar *N. pachyderma* (s), *N. pachyderma* (d), and *G. bulloides* $\delta^{18}O$ values, when the IRD peak is lower. Four minor IRD events (events a to d) in which various planktonic species showed a mix of similar and non-similar $\delta^{18}O$ values were also identified (Fig. 3A). For example, $\delta^{18}O$ values in *G. bulloides* and *N. pachyderma* (d) are similar but enriched for *N. pachyderma* (s) in IRD event d. The lack of convergence in $\delta^{18}O$ values among the planktonic foraminifera suggests that the mixing was restricted to only the uppermost water column during the minor IRD events in comparison to the HEs. In addition, the upper water masses are not well mixed between 19 and 35 ky B.P. and between 38.5 and 48.2 ky B.P., in comparison to the period of MIS 2 (Fig. 3B). Enriched $\delta^{18}O$ in *G. bulloides*, as compared with the *N. pachyderma* (d) between ~27.5 and 44 ky B.P., suggests the enhanced ventilation into the thermocline (*21*). As a result, subpolar-dwelling *G. bulloides* were transported and deposited at the core site.

A published data set from core CH69-K09 (*22*) from the northwestern margin of the IRD belt shows similar evidence for uniform water-mass conditions. *G. bulloides* and *N. pachyderma* (s) $\delta^{18}O$ values along this core are identical in HEs 1 to 4 and in H5a (*3*) (fig. S1). This implies that the incomplete evidence of uniform near-surface water in HEs in CHN82-20 may reflect its position at the southern margin of the IRD belt (*10*). To our knowledge, no other records on multiple species of planktonic foraminifera during HEs and other time intervals from the North

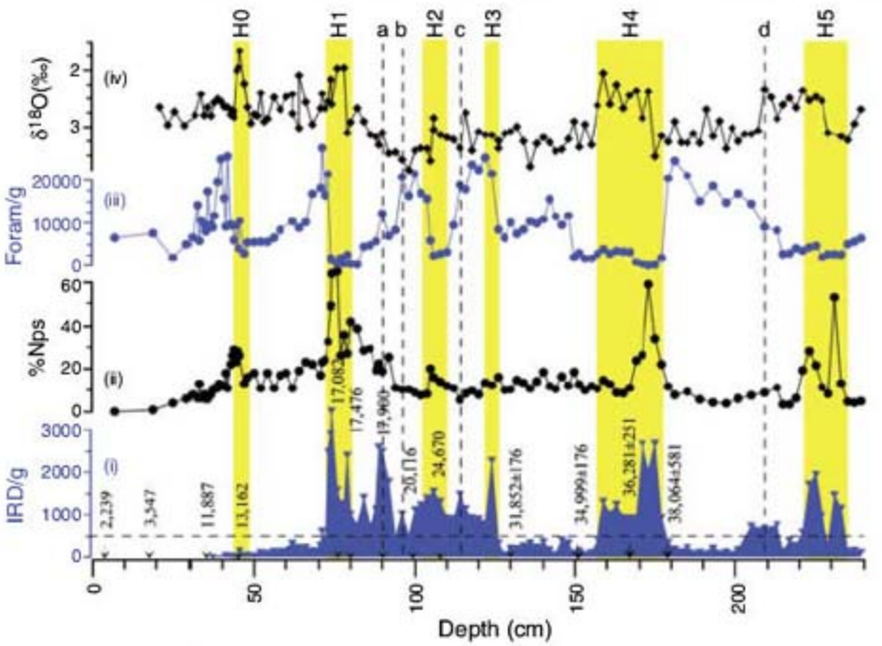


Fig. 2. Composite depth (in centimeters) in core CHN82-20 was achieved by patching trigger and piston cores , according to Boyle and Keigwin (*9*, *11*), as the upper part of the Holocene section was lost during coring. Values of (i) IRD per gram, (ii) % of *N. pachyderma* (s) (%Nps), (iii) total concentration of planktonic foraminifera per gram, and (iv) $\delta^{18}O$ in *N. pachyderma* (s) are plotted as a function of depth. Yellow bars and thin vertical dashed lines represent Heinrich and D/O ice-rafting layers, respectively. Downward arrows show ^{14}C dates converted to cal yr B.P. with calibration programs (*12*, *13*). Clear bubble-wall ash shards, correlated with the Vedde Ash Zone (*11*), were identified at 45 cm. The thin horizontal dashed line represents 500 IRD per gram (SOM text). H0, Heinrich event 0, a to d, D/O ice-rafting events.

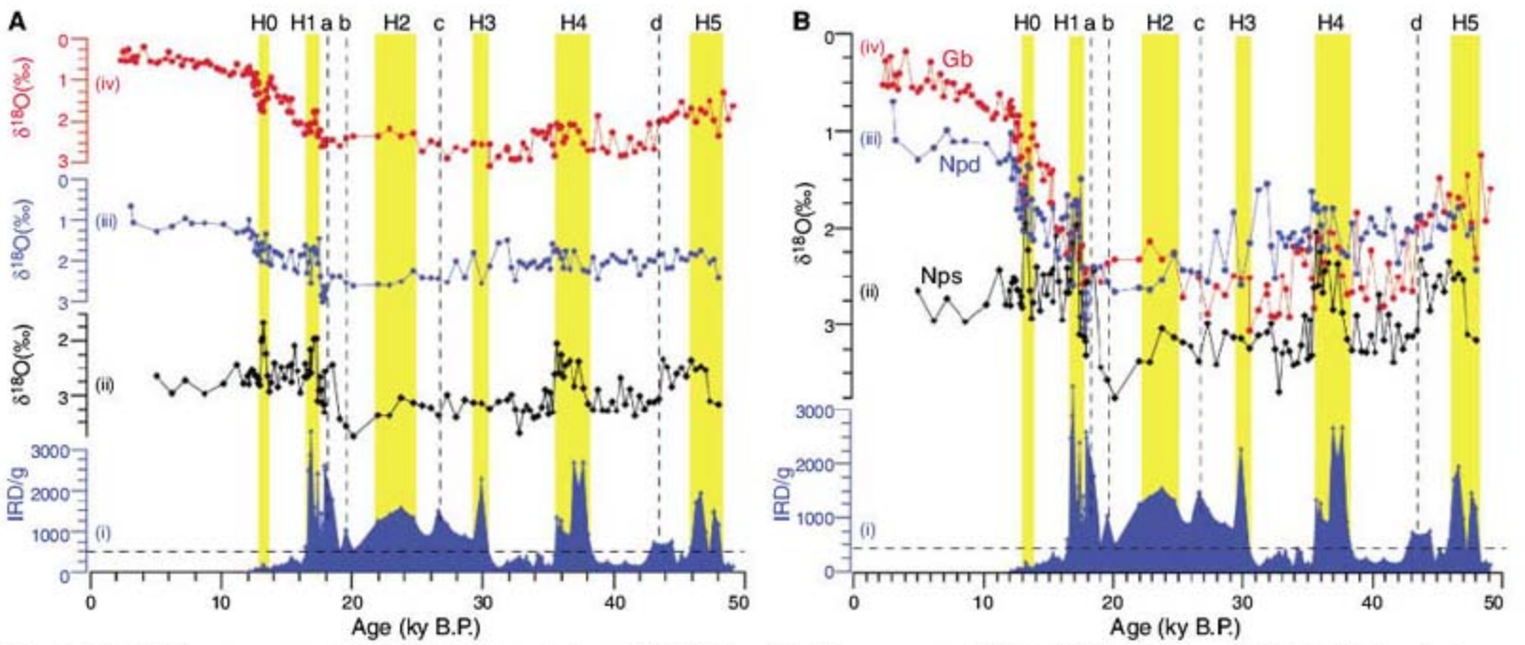


Fig. 3. (A) (i) IRD per gram and $\delta^{18}O$ in (ii) *N. pachyderma* (s), (iii) *N. pachyderma* (d), and (iv) *G. bulloides* in core CHN82-20 as a function of age (ky B.P.). Isotopic analyses were conducted on the 150- to 250-μm-size fraction to minimize any gametogenic effect. Yellow bars, vertical dashed lines, and the thin horizontal dashed line are the same as in Fig. 2. **(B)** (i) IRD per gram and $\delta^{18}O$ in (ii) *N. pachyderma* (s) (Nps), (iii) *N. pachyderma* (d) (Npd), and (iv) *G. bulloides* (Gb) in core CHN82-30 as a function of age (ky B.P.). $\delta^{18}O$ values on *N. pachyderma* (s), *N. pachyderma* (d), and *G. bulloides* were plotted with the same scale and exhibited identical values during H0, H1, and H4.

Atlantic are available, except a recent record from the Irish margin by Peck *et al.* (*23*). The authors recorded identical $\delta^{18}O$ *N. pachyderma* (s) and *G. bulloides* values during the LGM that were attributed to a continuous discharge of meltwater from the British Ice Sheet and year round mixing that homogenized the upper waters.

Several processes can create variability in the $\delta^{18}O$ of foraminifera. Seasonal variability was interpreted by Ganssen and Kroon (*19*) to explain why *G. bulloides* $\delta^{18}O$ was more positive than *G. inflata* $\delta^{18}O$ in the modern North Atlantic at 57°N, which was attributed to a later seasonal period of *G. bulloides* production further south. The uniform $\delta^{18}O$ of the foraminifera during HEs would require improbable ecological changes in preferred depth-habitat zones or in seasonal behavior if these values were not the result of uniform upper–water-mass conditions. Upwelling of ^{18}O-depleted water produced by brine-rejection at higher latitudes might affect the $\delta^{18}O$ of deeper-dwelling foraminifera; however, it is difficult to imagine it influencing $\delta^{18}O$ values in all three taxa. Without a decrease in temperature by ~2.5°C, it is impossible to lower the salinity by 0.8 per mil (‰) (*24*) (and by extension to lower $\delta^{18}O$ by 0.5‰) while remaining along the same isopycnal surface, which by itself would correspond to a 0.5‰ increase in the $\delta^{18}O$ of calcite. Because these effects cancel each other out, such a mechanism is inadequate to explain the anomalously low $\delta^{18}O$ values in all three planktonic foraminifera (Fig. 3B).

Intensified vertical mixing and deepening of the mixed layer during HEs is the mechanism remaining to explain the data. Atmospheric conditions directly influence the mixed layer through turbulence, and wind driven Langmuir circulation could be the prime driver of the turbulence (*25*, *26*). As a result, the upper ocean often becomes well mixed to depths as great as 600 m (*27*). Our $\delta^{18}O$ data from planktonic foraminifera that live at different depth ranges illustrate the extent of this process, suggesting that during the times of HEs the near-surface waters were homogenized by stronger mixing.

During the last glacial cycle, large ice sheets in the Northern Hemisphere and steeper meridional temperature gradients in the atmosphere must have reorganized atmospheric circulation. As a result, winter sea-ice cover extended further south, and glacial winds were stronger and more zonal (*28*, *29*). These winds would have intensified the vertical mixing and turbulence in the upper water masses. It is counterintuitive to visualize such a mechanism during HEs when the glacial North Atlantic was flooded with meltwater resulting in stronger stratification. However, our $\delta^{18}O$ data demonstrate that homogenization of upper water masses did occur, suggesting that this mechanism functioned at the core site. Additional evidence of this mechanism comes from the measurements of Ca^{+2} and Na^{+} ions derived from sea salt and continental dust in the Greenland ice core (*30*). Both Ca^{+2} and Na^{+} ions in the ice core show rapid increases from their ambient concentration during stadials. Abrupt, manyfold increases of these chemical species suggest that storminess during the glacial period caused stronger vertical mixing at the atmosphere/ocean boundary at the subpolar and subtropical fronts.

The question of why weaker homogenization of near-surface waters occurred during other D/O cycles not associated with HEs could be raised, because Greenland ice core data show similar patterns of glaciochemical species. One possibility is that unfavorable composition or insufficient volumes of meltwater were available to perturb the near-surface waters during these D/O ice-rafting cycles as the icebergs originated from the smaller ice sheets. Hence, even though the glacial climate was windier and stormier, the near-surface waters continued to be stratified.

References and Notes

1. H. Heinrich, *Quat. Res.* **29**, 143 (1988).
2. G. C. Bond *et al.*, *Nature* **365**, 143 (1993).
3. H. Rashid, R. Hesse, D. J. W. Piper, *Paleoceanography* **18**, 1077 (2003).
4. R. B. Alley, D. R. MacAyeal, *Paleoceanography* **9**, 503 (1994).
5. C. Huber *et al.*, *Earth Planet. Sci. Lett.* **243**, 504 (2006).
6. S. Manabe, R. J. Stouffer, *Nature* **378**, 165 (1995).
7. D. Rind *et al.*, *J. Geophys. Res.* **106**, 27335 (2001).
8. J. Flückiger, R. Knutti, J. W. C. White, *Paleoceanography* **21**, 1204 (2006).
9. E. A. Boyle, L. Keigwin, *Earth Planet. Sci. Lett.* **76**, 135 (1985/86).
10. W. F. Ruddiman, *Geol. Soc. Am. Bull.* **88**, 1813 (1977).
11. L. Keigwin, S. Lehman, *Paleoceanography* **9**, 185 (1994).
12. M. Stuiver, P. J. Reimer, *Radiocarbon* **35**, 215 (1993).
13. R. G. Fairbanks *et al.*, *Quat. Sci. Rev.* **24**, 1781 (2005).
14. H. Rashid, R. Hesse, D. J. W. Piper, *Earth Planet. Sci. Lett.* **208**, 319 (2003).
15. H. Rashid, E. Grosjean, *Paleoceanography* **21**, 1240 (2006).
16. J. J. Ottens, *Oceanol. Acta* **14**, 123 (1991).
17. W. G. Deuser, *J. Foraminifer. Res.* **17**, 14 (1987).
18. R. G. Fairbanks, P. H. Wiebe, A. W. Bé, *Science* **207**, 61 (1980).
19. G. Ganssen, D. Kroon, *J. Geol. Soc.* **157**, 693 (2000).
20. S. Mulitza, A. Dürkoop, W. Hale, G. Wefer, H. S. Niebler, *Geology* **25**, 335 (1997).
21. J. R. Luyten, A. J. Pedlosky, H. Stommel, *J. Phys. Oceanogr.* **13**, 192 (1983).
22. L. Labeyrie *et al.*, *AGU Monogr.* **112**, 77 (1999).
23. V. Peck *et al.*, *Earth Planet. Sci. Lett.* **243**, 476 (2006).
24. G. L. Pickard, W. J. Emery, *Descriptive Physical Oceanography* (Pergamon, Oxford, ed. 5, 1990).
25. S. K. Gulev, B. Barnier, H. Knochel, J.-M. Molines, M. Cottet, *J. Clim.* **16**, 3085 (2003).
26. K. Hanawa, T. Suga, in *Ocean-Atmosphere Interactions*, Y. Toba, Ed. (Kluwer Academic, Tokyo, 2003), pp. 63–109.
27. M. K. Robinson *et al.*, *Atlas of the North Atlantic-Indian Ocean Monthly Mean Temperatures and Mean Salinities of the Surface Layer* (U.S. Naval Oceanogr. Office Reference Publication 18, Washington, DC, 1978).
28. M. Sarnthein, U. Pflaumann, M. Weinelt, *Paleoceanography* **18**, 771 (2003).
29. H. Gildor, E. Tziperman, *Philos. Trans. R. Soc. London Ser. A* **361**, 1935 (2003).
30. P. A. Mayewski *et al.*, *Science* **263**, 1747 (1994).

31. We thank E. Goddard for helping to acquire part of the isotope data and D. J. W. Piper and B. P. Flower for discussions to improve an initial version of the manuscript. H.R. thanks Fonds pour la Formation de Chercheurs et l'Aide à la Recherche, Québec, for its support through a postdoctoral fellowship. E.A.B. was supported by grants from NSF and the Cambridge–Massachusetts Institute of Technology.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1146138/DC1
SOM Text
Fig. S1
Table S1
References

6 June 2007; accepted 11 September 2007
Published online 20 September 2007;
10.1126/science.1146138
Include this information when citing this paper.

Wasp Gene Expression Supports an Evolutionary Link Between Maternal Behavior and Eusociality

Amy L. Toth,[1*] Kranthi Varala,[2] Thomas C. Newman,[1] Fernando E. Miguez,[2] Stephen K. Hutchison,[3] David A. Willoughby,[3] Jan Fredrik Simons,[3] Michael Egholm,[3] James H. Hunt,[4] Matthew E. Hudson,[2] Gene E. Robinson[1,5]

The presence of workers that forgo reproduction and care for their siblings is a defining feature of eusociality and a major challenge for evolutionary theory. It has been proposed that worker behavior evolved from maternal care behavior. We explored this idea by studying gene expression in the primitively eusocial wasp *Polistes metricus*. Because little genomic information existed for this species, we used 454 sequencing to generate 391,157 brain complementary DNA reads, resulting in robust hits to 3017 genes from the honey bee genome, from which we identified and assayed orthologs of 32 honey bee behaviorally related genes. Wasp brain gene expression in workers was more similar to that in foundresses, which show maternal care, than to that in queens and gynes, which do not. Insulin-related genes were among the differentially regulated genes, suggesting that the evolution of eusociality involved major nutritional and reproductive pathways.

A major challenge in biology is to understand the evolution of animal society in molecular terms. Eusociality is the most extreme form of cooperation, typified by individuals that care for siblings rather than reproduce themselves, i.e., "workers." The evolution

of eusociality has been ascribed to kin or colony-level selection (*1*, *2*), but these explanations do not specify mechanistic routes.

It has long been suggested (*3*–*5*) that sibling care by hymenopteran (ant, bee, wasp) workers evolved from maternal care, which involves provisioning brood by foraging for food and then feeding them. According to this idea, two principal behaviors exhibited by solitary Hymenoptera, reproduction (egg-laying) and maternal care (brood provisioning), became uncoupled during the early stages of social evolution (*6*), and these behaviors eventually occurred in separate castes, queens and workers, respectively (*7*). Linksvayer and Wade (*8*) added a molecular dimension to this idea by predicting that sibling care and maternal care behaviors should be regulated by similar patterns of gene expression.

We used *Polistes* paper wasps to test Linksvayer and Wade's idea. *Polistes* are primitively eusocial, which means that although individuals specialize as either workers or reproductive individuals, these two castes are less distinct than in advanced eusocial species. In *Polistes*, both workers and reproductives display provisioning behavior, but at different points in the life of a colony. Advanced eusocial insects, by contrast, have morphologically distinct queen and worker castes, and in some species, such as the honey bee, queens no longer exhibit any maternal care, which precludes comparing the molecular basis of sibling and maternal care. Primitively eusocial insects like *Polistes* afford the opportunity to explore the molecular basis of maternal and worker behavior within a single species.

We measured brain gene expression in 87 individuals from four distinct behavioral groups of females from naturally occurring colonies of the temperate species *Polistes metricus* (Fig. 1A). Foundresses are females that establish new colonies in the spring, often as solitary individuals. Foundresses exhibit both reproductive (egg-laying) and maternal (foraging and brood-feeding) behavior. After rearing a first generation of female brood that develop into workers, successful foundresses become queens and cease caring for brood. Workers take over provisioning the brood—their siblings—by foraging for food and then feeding them; workers show little, if any, reproductive behavior. By contrast, queens focus exclusively on reproductive behavior. Gynes are reared late in the season; they engage in no reproductive or maternal care behavior (*9*). After successfully mating, gynes overwinter and then become foundresses (*10*). We hypothesized that brain gene expression patterns in *P. metricus* workers and foundresses should be most similar to each other from among these four groups, because they both show brood provisioning behavior despite their different reproductive status. Alternatively, if brain gene expression more closely reflects reproductive behavior, expression in foundresses and queens should be most similar to each other.

Social behavior is a complex and polygenic trait, so an appropriate test of the idea that maternal and worker behavior share a common molecular basis requires analysis of multiple genes in different pathways. But *Polistes* wasps, though venerable models for studies of social evolution (*11*, *12*), have until recently lacked genomic sequence information (*13*). To provide a ready source of test genes for quantitative reverse transcription–polymerase chain reaction analysis, we used 454 sequencing to obtain 45 megabases (Mb) in 391,157 cDNA sequence fragments from the *P. metricus* brain transcriptome (*14*). We were interested to see whether this low-cost, high-throughput sequencing method would be successful for this purpose, despite short se-

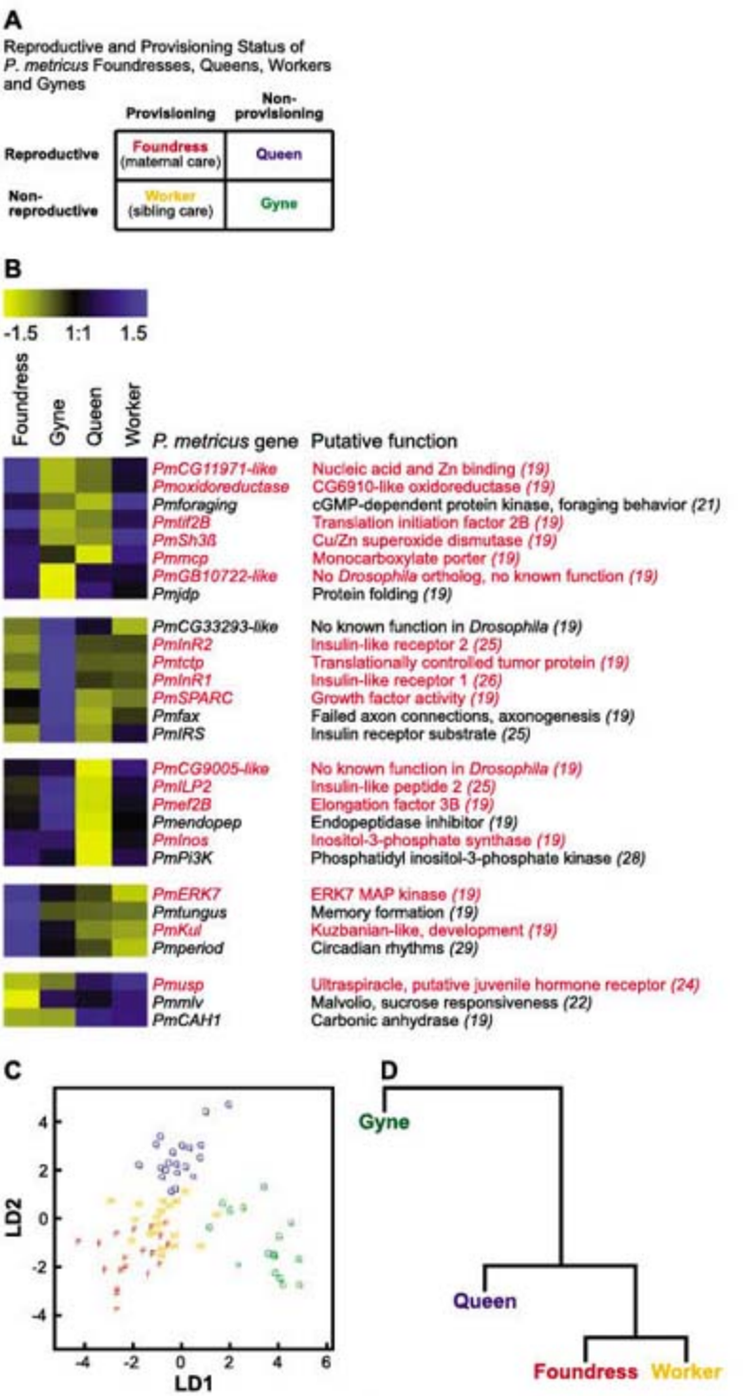


Fig. 1. *P. metricus* wasp brain gene expression analysis tests the prediction that maternal and worker (eusocial) behavior share a common molecular basis. **(A)** Similarities and differences in reproductive and brood provisioning status for the four behavioral groups analyzed in this study: foundresses (n = 22), gynes (n = 20), queens (n = 23), and workers (n = 22). Each individual wasp (total of 87) was assigned to a behavioral group on the basis of physiological measurements (*14*). **(B** to **D)** Results for 28 genes selected for their known involvement in worker (honey bee) behavior. (B) Heatmap of mean expression values by group and a summary of analysis of variance (ANOVA) results for each gene. Genes were clustered by K-means clustering (*37*); those in red showed significant differences (ANOVA, $P < 0.05$; table S1) between the behavioral groups. *P. metricus* gene names were assigned on the basis of orthology to honey bee genes (reference in parentheses); putative functions were assigned on the basis of similarity to *Drosophila melanogaster* genes. (C) Results of linear discriminant analysis show that foundress and worker brain profiles are more similar to each other than to the other groups. (D) Results of hierarchical clustering show the same result (based on group mean expression value for each gene). Four genes (*PmVg*, *Pmg5sd*, *PmGlyP*, and *PmRfaBp*) were excluded from these analyses because they showed high levels of expression in tissue adjacent to the brain (fig. S2); results for all three analyses were similar with and without these four genes (fig. S3).

[1]Department of Entomology and Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA. [2]Department of Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA. [3]454 Life Sciences, Branford, CT 06405, USA. [4]Department of Biology, University of Missouri at St. Louis, St. Louis, MO 63121, USA. [5]Neuroscience Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA.

*To whom correspondence should be addressed. E-mail: amytoth@uiuc.edu

quence read lengths (average of 120 bp) and an estimated 100- to 150-million-year divergence time between *P. metricus* and the honey bee, *Apis mellifera* (*15*), the most closely related species with a sequenced genome to use as reference (*16*).

We generated a map of the honey bee genome combined with known transcripts and their relative abundance in the combined bee expressed sequence tag (EST) data sets (*16*–*18*). *P. metricus* transcript fragments predicted to encode proteins orthologous to those encoded by *A. mellifera* genes were plotted on the map according to the number of fragments identified for a particular locus; matches were found for 39% of all honey bee mRNAs. The relative abundance of *P. metricus* sequence fragments corresponded well with the abundance of *A. mellifera* ESTs for the respective loci (Fig. 2). The combined *P. metricus*–*A. mellifera* transcriptome data set was then used to select the genes for this study.

Prior information allowed us to focus on genes implicated in honey bee foraging and provisioning behavior, rather than a set of randomly chosen genes that might be less informative. We selected 32 genes (Fig. 1B and table S1) from the *P. metricus* EST set that are orthologs of *A. mellifera* genes known to be associated in some way with worker bee behavior, based on results from studies with microarrays (22 genes) (*19*, *20*) and candidate genes (10 genes) (*21*–*29*). Twenty-two of the genes have been shown by microarray analysis to be both differentially expressed in the brains of honey bees engaged in foraging or feeding brood [on the list of the "top 100" genes most consistently associated with bee foraging behavior (*19*)] and regulated by juvenile hormone (*20*), which also causes worker bee foraging behavior (*30*). Five candidate genes are differentially expressed in honey bees engaged in foraging or feeding brood (*21*–*24*, *29*), three of which also have been shown to play causal roles in the regulation of worker bee foraging behavior (*21*, *22*, *31*). Five additional candidate genes involved in insulin signaling were selected because this pathway is implicated in honey bee queen-worker caste determination (*25*, *26*, *32*) and worker foraging behavior (*27*, *28*). Patterns of gene expression in *P. metricus* were not used as criteria for gene selection.

There was a robust association between individual wasp brain gene expression and naturally occurring behavioral differences among the wasp groups. Leave-one-out cross-validation analysis (*19*) resulted in 68, 69, 70, and 47% correct assignments to the foundress, gyne, queen, and worker groups, respectively. For the less conservative resubstitution method (*33*), the results were 89, 100, 100, and 95%. The predictions from both classification methods were significantly better than random (Chi-square tests, $P < 0.0001$, 25% expected). This honey bee–derived gene set thus demonstrates extensive brain regulation across the four wasp groups, making it an informative set to explore the molecular relationship between maternal and worker behavior in *P. metricus*.

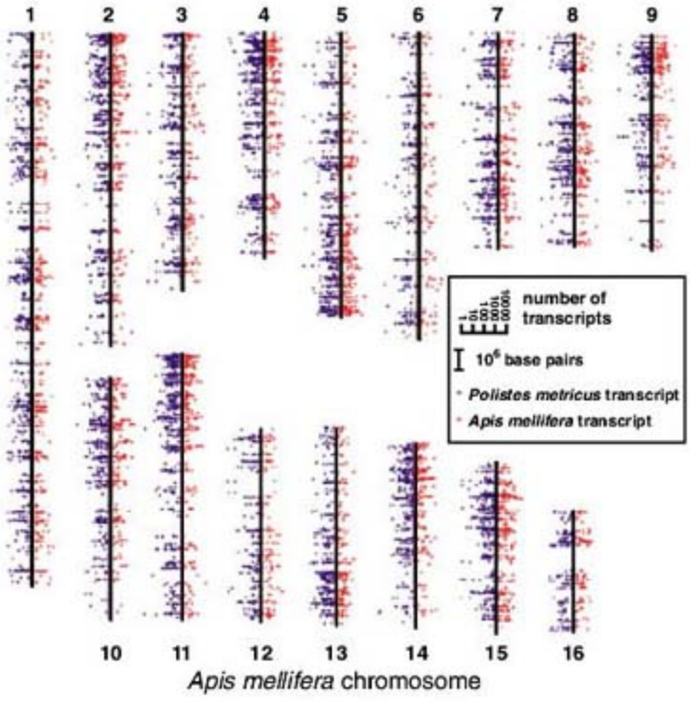


Fig. 2. A representation of *P. metricus* brain transcripts overlaid on a honey bee genome template (*16*) shows wide coverage and similar transcript abundance for *P. metricus* relative to known honey bee transcripts. *P. metricus* brain cDNA sequence fragments were matched as predicted proteins to *A. mellifera* transcripts with experimental support (known cDNA or EST sequences). *A. mellifera* transcripts (red points, right of axis) and their closest *P. metricus* orthologs from our survey (blue points, left of axis) were then mapped to the corresponding genomic locus in the *A. mellifera* genome. The vertical lines represent *A. mellifera* chromosomes 1 to 16. The distance of each point from the midline is proportional to the logarithm of the abundance of the mRNA (the number of sequences for each *P. metricus* or *A. mellifera* transcript corresponding to the *A. mellifera* gene at that locus) (*16*–*18*). *P. metricus* orthologs were obtained for a total of 3017 *A. mellifera* transcripts. The *P. metricus* transcriptome data contained putative orthologs for 39% of known *A. mellifera* mRNAs. An additional 252,556 transcript sequence fragments obtained from *P. metricus* did not have a clearly orthologous transcript in *A. mellifera*.

Sixty-two percent of the genes in the gene set were differentially regulated in *P. metricus* as a function of reproductive or provisioning behavior (Fig. 1B and table S1). Multivariate analysis of variance showed that brain gene expression varied significantly with reproduction ($F = 3.28$, $P = 0.0002$) and provisioning ($F = 4.76$, $P < 0.0001$), with a significant provisioning × reproduction interaction ($F = 2.48$, $P = 0.002$). Three out of the five insulin-related genes showed significant associations with provisioning and/or reproductive behavior, consistent with known nutritional effects on behavior and physiology in honey bees and other social insects (*34*).

Three statistical analyses demonstrated that brain gene expression for worker wasps was more similar to that of maternal females (foundresses) than to that of females not showing maternal care (queens and gynes). First, K-means clustering (Fig. 1B and fig. S1) revealed five clusters of coexpressed genes. The first cluster contained genes ($n = 8$) that showed coexpression in foundresses and workers compared to queens and gynes. The second cluster of genes ($n = 7$) was mainly characterized by up-regulation in gynes, and the third ($n = 6$) by down-regulation in queens, but in both of these clusters, foundresses and workers also showed patterns of expression that were similar to each other (Fig. 1B and fig. S1).

The second statistical analysis, linear discriminant (LD) analysis, also showed similarities between foundress and worker brain gene expression (Fig. 1C). A plot of LD1 versus LD2 (which accounted for 90% of the variation in brain gene expression across all four groups) revealed group-specific expression patterns, but foundresses and workers showed the greatest overlap. This is consistent with the poorer performance of classification methods (described above) for those two groups; overlap in gene expression patterns made them difficult to distinguish from each other. Gynes, which engage in neither reproductive nor provisioning behavior, were the most distinct group. The third statistical analysis, hierarchical clustering by group, supported the patterns found in the other two analyses—brain gene expression of workers and foundresses was most similar, and that of gynes was most divergent (Fig. 1D).

There are marked temporal changes in brain gene expression as females shift from foundress to queen status, i.e., from maternal to reproductive behavior. These findings demonstrate heterochronic expression of genes associated with maternal behavior, a form of plasticity that is considered to be necessary for the evolution of worker behavior (*8*). They also reflect the apparent modularity of egg-laying and brood provisioning behavior and their underlying regulatory networks; this type of modularity also is thought to be important in the evolution of novel traits (*35*).

We used the honey bee genome, together with "next-generation" sequencing technology, to rapidly bring genomics to the relatively closely

related wasp *P. metricus*; this is an early example of the utility of 454 sequencing for transcriptomics (*36*). Our results demonstrate that it is possible to use species that have had their genomes sequenced as "hubs" to efficiently generate genomic resources for clusters of related species that might each be especially well suited to address particular evolutionary problems. This "hub and spokes" approach should enable genomics to be deployed for a broader range of species than is currently being done, until whole-genome sequencing of eukaryote genomes becomes routine.

References and Notes

1. E. O. Wilson, B. Hölldobler, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 13367 (2005).
2. L. Lehmann, L. Keller, *J. Evol. Biol.* **19**, 1365 (2006).
3. W. M. Wheeler, *The Social Insects: Their Origin and Evolution* (Harcourt, Brace, New York, 1928).
4. H. E. Evans, M. J. West-Eberhard, *The Wasps* (Univ. of Michigan Press, Ann Arbor, MI, 1970).
5. J. H. Hunt, *Evolution* **53**, 225 (1999).
6. M. J. West-Eberhard, in *Natural History and Evolution of Paper-Wasps*, S. Turillazzi, M. J. West-Eberhard, Eds. (Oxford Univ. Press, New York, 1996), pp. 290–317.
7. E. O. Wilson, *The Insect Societies* (Belknap, Cambridge, MA, 1971).
8. T. A. Linksvayer, M. J. Wade, *Q. Rev. Biol.* **80**, 317 (2005).
9. M. J. West-Eberhard, *Misc. Publ. Mus. Zool. Univ. Mich.* **140**, 1 (1969).
10. J. H. Hunt, *The Evolution of Social Wasps* (Oxford Univ. Press, New York, 2007).
11. S. Turillazzi, M. J. West-Eberhard, Eds., *Natural History and Evolution of Paper-Wasps* (Oxford Univ. Press, New York, 1996).
12. H. K. Reeve, in *The Social Biology of Wasps*, K. G. Ross, R. W. Matthews, Eds. (Cornell Univ. Press, Ithaca, NY, 1991).
13. S. Sumner, J. J. M. Pereboom, W. C. Jordan, *Proc. R. Soc. London B Biol. Sci.* **273**, 19 (2006).
14. Materials and methods are available as supporting material on *Science* Online.
15. B. N. Danforth, S. G. Brady, S. D. Sipes, A. Pearson, *Syst. Biol.* **53**, 309 (2004).
16. Honey Bee Genome Sequencing Consortium, *Nature* **443**, 931 (2006).
17. F. M. F. Nunes *et al.*, *BMC Genomics* **5**, 84 (2004).
18. C. W. Whitfield *et al.*, *Genome Res.* **12**, 555 (2002).
19. C. W. Whitfield, A. M. Cziko, G. E. Robinson, *Science* **302**, 296 (2003).
20. C. W. Whitfield *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 16068 (2006).
21. Y. Ben-Shahar, A. Robichon, M. B. Sokolowski, G. E. Robinson, *Science* **296**, 741 (2002).
22. Y. Ben-Shahar, N. L. Dudek, G. E. Robinson, *J. Exp. Biol.* **207**, 3281 (2004).
23. G. V. Amdam, K. Norberg, M. K. Fondrk, R. E. Page, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 11350 (2004).
24. A. R. Barchuk, R. Maleszka, Z. L. P. Simoes, *Insect Mol. Biol.* **13**, 459 (2004).
25. D. E. Wheeler, N. Buck, J. D. Evans, *Insect Mol. Biol.* **15**, 597 (2006).
26. M. Corona *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 7128 (2007).
27. S. A. Ament, R. A. Verlarde, G. E. Robinson, *Society for Neuroscience Itinerary Planner and Abstract Viewer*, "Neuropeptide Y signaling and nutritionally mediated social behavior in the honey bee" (2006).
28. G. J. Hunt *et al.*, *Naturwissenschaften* **94**, 247 (2007).
29. G. Bloch, D. P. Toma, G. E. Robinson, *J. Biol. Rhythms* **16**, 444 (2001).
30. G. Bloch, D. E. Wheeler, G. E. Robinson, in *Hormones, Brain and Behavior*, D. W. Pfaff, A. Arnold, A. Etgen, S. Fahrbach, R. Rubin, Eds. (Elsevier Science, St. Louis, MO, 2002), vol. 3, pp. 195–235.
31. M. Nelson, K. Ihle, M. K. Fondrk, R. E. Page, G. V. Amdam, *PLoS Biol.* **5**, e62 (2007).
32. A. Patel *et al.*, *PLoS One* **2**, e509 (2007).
33. W. N. Venables, B. D. Ripley, *Modern Applied Statistics with S* (Springer, New York, ed. 4, 2002).
34. A. L. Toth, S. Kantarovich, A. F. Meisel, G. E. Robinson, *J. Exp. Biol.* **208**, 4641 (2005).
35. M. J. West-Eberhard, *Developmental Plasticity and Evolution* (Oxford Univ. Press, New York, 2003).
36. M. E. Hudson, *Mol. Ecol. Notes*, 10.1111/j.1471-8286.2007.02019.x (2007).
37. A. Sturn, J. Quackenbush, Z. Trajanoski, *Bioinformatics* **18**, 207 (2002).

38. We thank A. S. Escalante, A. Bowling, S. Kantarovich, K. J. Bilof, and S. Buck for assistance in the field; D. Schejbal and S. Buck for permission to collect wasps at field sites owned by the University of Illinois; A. S. Escalante and K. J. Bilof for physiological measurements; M. T. Henshaw for microsatellite analyses; R. A. Gibbs for strategic assistance; C. W. Whitfield for assistance with gene identification; R. Rego for brain dissections and RNA extractions; Y. Li, Y. Lu, and S. Zhong for assistance with statistical analysis; E. L. Hadley for assisting with figure preparation; and M. B. Sokolowski, H. M. Robertson, C. M. Grozinger, C. W. Whitfield, M. R. Berenbaum, S. A. Cameron, J. L. Beverly, members of the Robinson laboratory, and members of the University of Illinois Social Insect Training Initiative for constructive comments on the manuscript. Supported by the Illinois Sociogenomic Initiative and NSF grant IOS-0641431 (G.E.R.). The individual *P. metricus* sequences and flowgram data have been uploaded to NCBI Trace Archive, TI range 1888756160 to 1889135944.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1146647/DC1
Materials and Methods
Figs. S1 to S3
Tables S1 and S2
References

18 June 2007; accepted 19 September 2007
Published online 27 September 2007;
10.1126/science.1146647
Include this information when citing this paper.

JMJD6 Is a Histone Arginine Demethylase

Bingsheng Chang, Yue Chen, Yingming Zhao, Richard K. Bruick*

Arginine methylation occurs on a number of proteins involved in a variety of cellular functions. Histone tails are known to be mono- and dimethylated on multiple arginine residues where they influence chromatin remodeling and gene expression. To date, no enzyme has been shown to reverse these regulatory modifications. We demonstrate that the Jumonji domain–containing 6 protein (JMJD6) is a JmjC-containing iron- and 2-oxoglutarate–dependent dioxygenase that demethylates histone H3 at arginine 2 (H3R2) and histone H4 at arginine 3 (H4R3) in both biochemical and cell-based assays. These findings may help explain the many developmental defects observed in the JMJD6$^{-/-}$ knockout mice.

Iron- and 2-oxoglutarate–dependent dioxygenases have been shown to oxidize a variety of substrates including metabolites, nucleic acids, and proteins (*1*). A candidate dioxygenase, JMJD6, shares extensive sequence and predicted structural homology with an asparaginyl hydroxylase (*2*, *3*) as well as the JmjC domains found in several histone lysine demethylases (fig. S1A) (*4*–*8*). Given the predicted conservation of structural elements and key residues (*9*–*11*), it is likely that JMJD6 retains an analogous catalytic activity. Here we report in vitro and in vivo data that clearly indicate that JMJD6 functions as an arginine demethylase.

To test whether JMJD6 demethylates the N-terminal tails of histone H3 or H4, we incubated bulk histones with JMJD6 in the presence of Fe(II), 2-oxoglutarate, and ascorbate (*12*). Antibodies specific for various methylated sites on histones H3 and H4 were used to assess demethylation. Although no lysine demethylation was observed, a substantial reduction in H3R2me2 and H4R3me2 was observed in the presence of JMJD6 compared with buffer alone (Fig. 1A). These effects were site-specific as no changes in dimethylarginine were seen at positions H3R17 or H3R26. Previously, no enzyme had been shown to reverse regulatory arginine methylation, although deiminases can convert methylarginine to citrulline via demethylimination (*13*, *14*). However, the requisite chemistry is analogous to that demonstrated for demethylation of alkylated nitrogens by other dioxygenases (fig. S1C).

To investigate the preference for the substrate methylation state, we used antibodies specific for either mono- or dimethylated (symmetric) H4R3 (Fig. 1B). The recombinant JMJD6 was able to demethylate H4R3me2 when either heterogeneous bulk histones or synthetic peptides encompassing the N-terminal 30 residues of histone H4 were used as substrates (Fig. 1C). To a lesser extent, JMJD6 could also demethylate H4R3me1-containing substrates (Fig. 1C). Mutation of the residues predicted to mediate Fe(II) binding (mut JMJD6) prevented demethylation (Fig. 1C).

Department of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390–9038, USA.

*To whom correspondence should be addressed. E-mail: richard.bruick@utsouthwestern.edu

To confirm that JMJD6 promotes oxidative demethylation of arginine residues, we examined product formation. Appearance of the H3R4me1 product from the dimethylated peptide substrate (p2meR3H4) was observed in the presence of wild-type (wt) recombinant JMJD6, but not the inactive (mut) variant (Fig. 2A). Product formation was dependent on the presence of Fe(II), 2-oxoglutarate, and ascorbate (Fig. 2B), and activity was subject to saturation by substrate (fig. S2C). Generation of a second product, formaldehyde, was measured with recombinant histone H3 and H4 proteins radiolabeled with ^{3}[H]CH_3-. Upon demethylation, the released ^{3}H-formaldehyde was converted to radiolabeled 3,5-diacethyl-1,4-dihydrolutidine and quantitated. Again, only wt JMJD6 could liberate ^{3}H-formaldehyde from histones H3 and H4 methylated on arginine (Fig. 2C), but mut JMJD6 could not liberate ^{3}H-formaldehyde from H3 methylated on K36 (Fig. 2D).

To directly demonstrate arginine demethylation, we used matrix-assisted laser desorption/ionization–time-of-flight (MALDI-TOF) mass spectrometry (MS) to analyze the reaction products. The 30–amino acid histone H4 peptide synthesized with symmetric dimethylarginine (SDMA) at the third position (p2meR3H4) was incubated with buffer alone, wt JMJD6, or inactive (mut) JMJD6. The resulting peptides were immunoprecipitated with the α-H4R3me1 antibody specific for the monomethylated product and analyzed by MALDI-TOF mass spectrometry. Incubation of the p2meR3H4 peptide with wt JMJD6, but not the inactive mutant, led to mass shift of the peptide from 3129 to 3115 daltons, suggesting a loss of one methyl group (14 daltons) (Fig. 3A). Similarly, formation of the H3R2me1 product was observed by MS upon incubation of the p2meR2H3 peptide with wt JMJD6 (fig. S3). The possible formation of the fully demethylated peptide product could not be ascertained under these assay conditions because it was first necessary to enrich the reaction products by immunoprecipitation with the α-H4R3me1 antibody.

In addition to the expected demethylated (H4R3me1) product, two additional products (+16 and +32 daltons) were observed that likely represent additional oxidation of the monomethylated product. To further validate the demethylation product and identify additional modification sites, we analyzed the electron transfer dissociation (ETD) MS/MS spectra of the products (Fig. 3B, top panel). Although all of the C ions indicated the loss of a methyl group (−14 daltons) upon incubation with JMJD6, the data also revealed that the 3131.82-dalton peak contains a mono-oxidation product of p1meR3H4 with modification preferentially occurring at lysine 8 (Fig. 3B, middle panel). The 3147.81-dalton product was mainly oxidized on lysine 8 and lysine 5 (Fig. 3B; bottom panel). Such oxidation in vitro was also observed with the histone H3 peptide substrate (figs. S3 and S5). Although the physiological significance of the observed lysine oxidation is unknown, it is nevertheless clear that JMJD6 can effect arginine demethylation in vitro.

Unlike previously characterized JmjC-containing lysine demethylases, the JMJD6 catalytic domain is not accompanied by recognizable domains that help target these enzymes to histones (*15*), perhaps contributing to the low activity of our recombinant protein in vitro. It is possible that JMJD6 is recruited to specific chromatin sites through interactions with other proteins. To determine whether JMJD6 could efficiently promote arginine demethylation in the context of living cells, we transfected V5-tagged expression constructs encoding either wt JMJD6 or an inactive variant that lacks one of the Fe(II) ligands (H187A; mut). Indirect immunofluorescence staining with antibodies recognizing the V5 tag or various histone arginine methylation sites

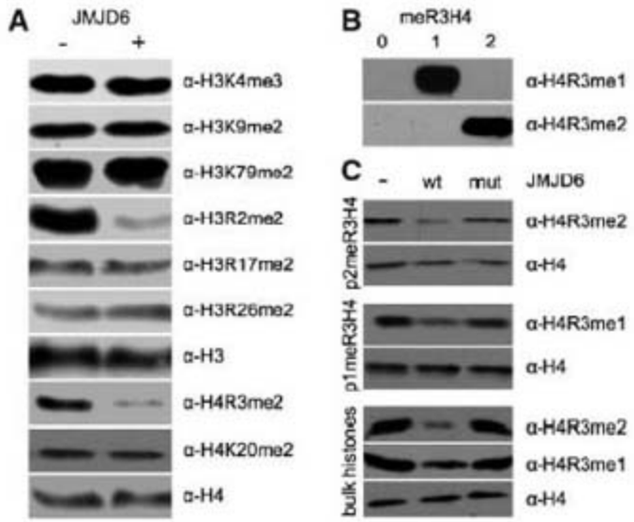


Fig. 1. JMJD6 is a putative Fe(II)- and 2-oxoglutarate–dependent dioxygenase. (**A**) JMJD6 demethylates H3R2me2 and H4R3me2. Bulk histones were incubated in the presence (+) or absence (–) of purified recombinant JMJD6. Antibodies (Abcam) specific for the indicated histone methylation sites were used to detect loss of these modifications by Western blot analysis. Recognition of the indicated methylated sites was confirmed with blocking peptides (fig. S2A). Total amounts of histone H3 and H4 are shown as loading controls. Similar results were obtained with antibodies from another manufacturer (fig. S2B). (**B**) The α-H4R3me1 and α-H4R3me2 antibodies specifically recognize synthetic peptide substrates, where R3 contains one (1) or two (2) methyl groups, respectively. (**C**) JMJD6 demethylates both mono- and dimethylarginine residues. Bulk histones or histone H4 peptides were synthesized with symmetric dimethylarginine (p2meR3H4) or monomethylarginine (p1meR3H4) and incubated in the absence (–) or presence of wt JMJD6 protein or a catalytically inactive (mut) JMJD6 (H187A; D189A; H273A). Demethylation was assessed by Western blot analysis.

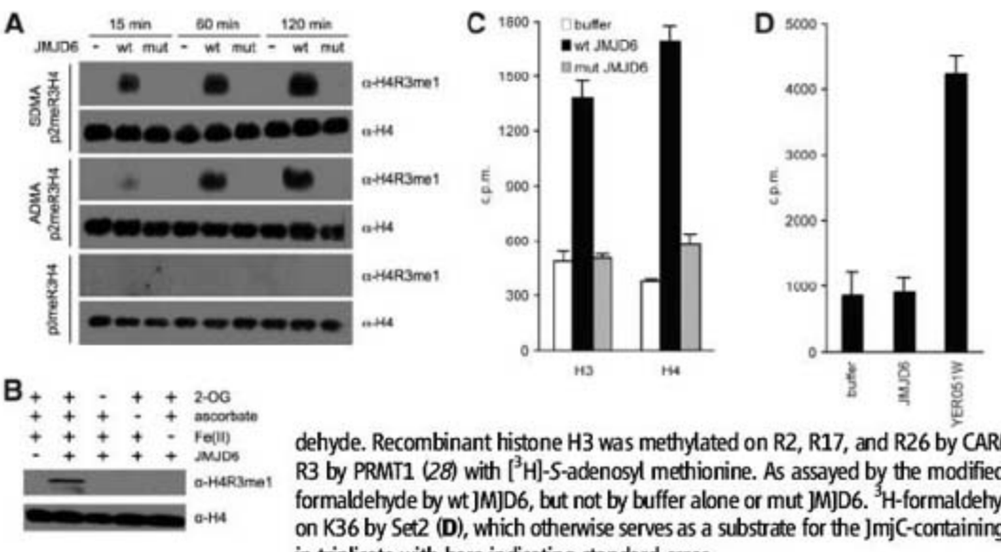


Fig. 2. JMJD6 is a Fe(II)- and 2-oxoglutarate–dependent dioxygenase. (**A**) Formation of the H4R3me1 product from symmetric (SDMA) or asymmetric (ADMA) dimethylarginine containing p2meR3H4 substrates. Peptide substrates derived from histone H4 containing two or zero methyl groups on R3 were incubated in the absence (–) or presence (+) of wt JMJD6 protein or a catalytically dead (mut) JMJD6 (H187A; D189A; H273A). The α-H4R3me1 antibody was used to detect formation of this product by Western blot analysis. (**B**) Formation of the demethylated H4R3me1 product from the p2meR3H4 substrate by JMJD6 requires Fe(II), ascorbate, and 2-oxoglutarate (2-OG). (**C**) JMJD6-mediated arginine demethylation generates formaldehyde. Recombinant histone H3 was methylated on R2, R17, and R26 by CARM1 (*28*), and recombinant histone H4 was methylated on R3 by PRMT1 (*28*) with [^{3}H]-*S*-adenosyl methionine. As assayed by the modified Nash method (*29*), ^{3}H-methyl groups were liberated as formaldehyde by wt JMJD6, but not by buffer alone or mut JMJD6. ^{3}H-formaldehyde is not produced by JMJD6 from histone H3 methylated on K36 by Set2 (**D**), which otherwise serves as a substrate for the JmjC-containing lysine demethylase Yer051w (*4*). Assays were performed in triplicate with bars indicating standard error.

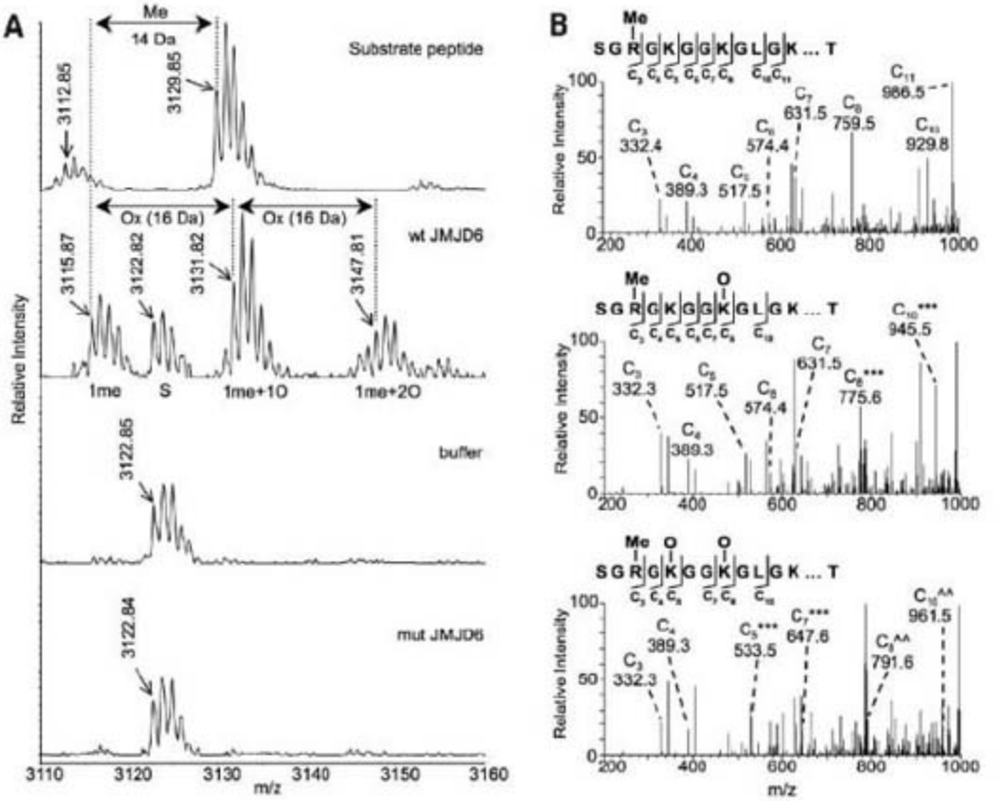


Fig. 3. Analysis of demethylation products by mass spectrometry. **(A)** JMJD6 demethylates p2meR3H4 to the monomethylated product (1me). The p2meR3H4 peptide substrate [expected protonated molecular mass (M + 1) = 3129.6 daltons] was incubated with buffer alone or JMJD6 (wt or mut). Products were immunoprecipitated with the α-H4R3me1 antibody before analysis. A p1meR3H4 peptide synthesized with a stable isotope of leucine (containing six ^{13}C and one ^{15}N atoms) was added as an internal standard (S) before immunoprecipitation. The peak with *m/z* 3112.85 in the top panel is a deaminated impurity that was removed during immunoprecipitation. **(B)** ETD fragmentation of the demethylation products (+5 charged ions) indicates that in addition to demethylation, JMJD6 can promote oxidation of nearby lysine residues [1me + 1O or 1me + 2O in (A)]. The relevant ion fragments are labeled and the corresponding peptide positions are illustrated. Fragments containing a single oxidation modification are denoted by "***" and fragments containing two oxidation events are denoted by "^^". Shown are the partial (for full spectra, see fig. S4) MS/MS spectrum of the monomethyl product (top panel), the monomethyl product oxidized on K8 (middle panel), and the monomethyl product with oxidation of both K5 and K8 (bottom panel).

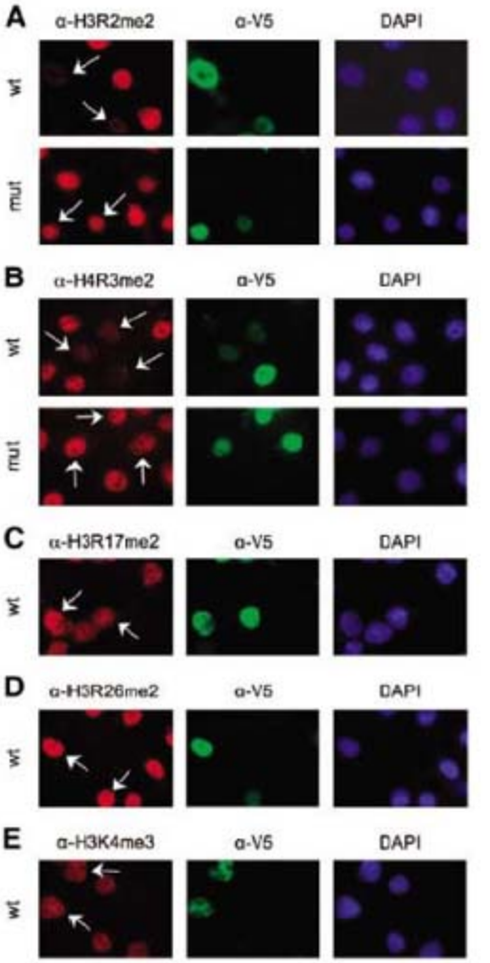


Fig. 4. JMJD6 promotes histone arginine demethylation in cultured HeLa cells. Transient overexpression of V5-tagged wild-type (wt), but not an inactive variant (mut) of JMJD6, reduces global amounts of H3R2me2 **(A)** and H4R3me2 **(B)**, but not H3R17me2 **(C)**, H3R26me2 **(D)**, or H3K4me3 **(E)**. DAPI (4′,6-diamidino-2-phenylindole) staining marks the location of nuclei in the field, and arrows indicate transfected cells.

revealed that cells overexpressing wt JMJD6, but not mut JMJD6, displayed a substantial reduction in global H3R2me2 (Fig. 4A) and H4R3me2 content (Fig. 4B), in line with the loss of staining observed in the presence of blocking peptides (figs. S6A and S6B). Consistent with the site selectivity observed with recombinant JMJD6 in vitro (Fig. 1A), JMJD6 overexpression had almost no effect on the global levels of H3R17me2 (Fig. 4C), H3R26me2 (Fig. 4D), or H3K4me3 (Fig. 4E). Collectively, these in vitro and cellular data both indicate that JMJD6 is a histone arginine demethylase.

JMJD6 was previously identified as the phosphatidylserine receptor responsible for recognizing apoptotic cells (*16*–*20*). Subsequent studies have challenged these conclusions (*21*–*23*), failing to confirm a role for JMJD6 in apoptotic cell clearance or phagocytosis (*23*, *24*). Instead, the findings presented here support a role for JMJD6 in the nucleus as a histone arginine demethylase. In conjunction with other modifications found on histone tails, methylarginine contributes to the histone code that mediates chromatin remodeling and gene expression (*25*). In addition, methylarginine residues have been found on a large number of nonhistone proteins (*26*), though it remains to be seen whether any are substrates for JMJD6. Data are emerging that arginine methylation plays an important role in cellular differentiation and proliferation during development (*25*). Knockdown or knockouts of JMJD6 in model organisms were accompanied by numerous developmental defects during embryogenesis (*18*–*20*, *23*) that may result from inappropriate methylation of histones and other proteins. Furthermore, reports that the Hypoxia-Inducible Factor (HIF) hydroxylases serve as cellular sensors by virtue of their substrate and cofactor requirements (*27*) raises the possibility that numerous cellular processes mediated by arginine or lysine demethylation could be directly regulated in response to dynamic changes in a cell's metabolic, environmental, or developmental status.

References and Notes

1. I. J. Clifton *et al.*, *J. Inorg. Biochem.* **100**, 644 (2006).
2. K. S. Hewitson *et al.*, *J. Biol. Chem.* **277**, 26351 (2002).
3. D. Lando *et al.*, *Genes Dev.* **16**, 1466 (2002).
4. Y. Tsukada *et al.*, *Nature* **439**, 811 (2006).
5. R. J. Klose *et al.*, *Nature* **442**, 312 (2006).
6. J. R. Whetstine *et al.*, *Cell* **125**, 467 (2006).
7. J. Christensen *et al.*, *Cell* **128**, 1063 (2007).
8. S. Iwase *et al.*, *Cell* **128**, 1077 (2007).
9. C. E. Dann III, R. K. Bruick, J. Deisenhofer, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 15351 (2002).
10. J. M. Elkins *et al.*, *J. Biol. Chem.* **278**, 1802 (2003).
11. Z. Chen *et al.*, *Cell* **125**, 691 (2006).
12. Materials and methods are available as supporting material on *Science* Online.
13. G. L. Cuthbert *et al.*, *Cell* **118**, 545 (2004).
14. Y. Wang *et al.*, *Science* **306**, 279 (2004).
15. R. J. Klose, E. M. Kallin, Y. Zhang, *Nat. Rev. Genet.* **7**, 715 (2006).
16. V. A. Fadok *et al.*, *Nature* **405**, 85 (2000).
17. X. Wang *et al.*, *Science* **302**, 1563 (2003).
18. J. R. Hong *et al.*, *Development* **131**, 5417 (2004).
19. Y. Kunisaki *et al.*, *Blood* **103**, 3362 (2004).
20. M. O. Li, M. R. Sarkisian, W. Z. Mehal, P. Rakic, R. A. Flavell, *Science* **302**, 1560 (2003).

21. M. Cikala *et al.*, *BMC Cell Biol.* **5**, 26 (2004).
22. P. Cui, B. Qin, N. Liu, G. Pan, D. Pei, *Exp. Cell Res.* **293**, 154 (2004).
23. J. Böse *et al.*, *J. Biol.* **3**, 15 (2004).
24. J. E. Mitchell *et al.*, *J. Biol. Chem.* **281**, 5718 (2006).
25. J. Wysocka, C. D. Allis, S. Coonrod, *Front. Biosci.* **11**, 344 (2006).
26. S. Pahlich, R. P. Zakaryn, H. Gehring, *Biochim. Biophys. Acta* **1764**, 1890 (2006).
27. A. Ozer, R. K. Bruick, *Nat. Chem. Biol.* **3**, 144 (2007).
28. A. J. Bannister, R. Schneider, T. Kouzarides, *Cell* **109**, 801 (2002).
29. T. Nash, *Biochem. J.* **55**, 416 (1953).
30. We thank T. Zhang (ThermoFisher Scientific) for performing the ETD analysis, H. Ball [University of Texas Southwestern (UTSW)] and K. Linse (University of Texas) for peptide synthesis, and K. Gardner (UTSW) for helpful comments. This work was supported by the Burroughs Wellcome Fund (R.K.B.), the Robert A. Welch Foundation (R.K.B and Y.Z.), and the NIH [CA115962 to R.K.B., CA107943 to Y.Z., and a Research Facilities Improvement Program Grant (C06-RR15437-01) from the National Center for Research Resources]. R.K.B. is the Michael L. Rosenberg Scholar in Medical Research.

Supporting Online Material
www.sciencemag.org/cgi/content/full/318/5849/444/DC1
Materials and Methods
Figs. S1 to S6
References

29 May 2007; accepted 5 September 2007
10.1126/science.1145801

Demethylation of H3K27 Regulates Polycomb Recruitment and H2A Ubiquitination

Min Gyu Lee,[1] Raffaella Villa,[2] Patrick Trojer,[3] Jessica Norman,[1] Kai-Ping Yan,[1] Danny Reinberg,[3] Luciano Di Croce,[2] Ramin Shiekhattar[1,2]*

Methylation of histone H3 lysine 27 (H3K27) is a posttranslational modification that is highly correlated with genomic silencing. Here we show that human UTX, a member of the Jumonji C family of proteins, is a di- and trimethyl H3K27 demethylase. UTX occupies the promoters of *HOX* gene clusters and regulates their transcriptional output by modulating the recruitment of polycomb repressive complex 1 and the monoubiquitination of histone H2A. Moreover, UTX associates with mixed-lineage leukemia (MLL) 2/3 complexes, and during retinoic acid signaling events, the recruitment of the UTX complex to *HOX* genes results in H3K27 demethylation and a concomitant methylation of H3K4. Our results suggest a concerted mechanism for transcriptional activation in which cycles of H3K4 methylation by MLL2/3 are linked with the demethylation of H3K27 through UTX.

The methylation of lysine residues on histones is often associated with either the activation [methylation of histone H3 lysine 4 (H3K4), H3K36, and H3K79] or repression (methylation of H3K9, H3K27, and H4K20) of transcription (*1*, *2*). Methylation offers an additional level of control by permitting single, double, and triple modification of the same lysine residues (*1*, *2*), resulting in differential regulation of many cellular processes (*1*–*3*). The Methylation of H3K27 is implicated in X chromosome inactivation, imprinting, stem cell maintenance, circadian rhythms, and cancer (*4*–*6*) and is carried out by Enhancer of zeste homolog 2 methyltransferase, a component of the mammalian polycomb repressive complexes (PRCs), including PRC2 (*7*–*9*). Tri- and dimethyl H3K27 are enriched on inactive X chromosomes, as well as at promoter regions of inactive genes (*2*, *10*, *11*). Here, we describe the characterization of UTX, a Jumonji C (JmjC)–domain-containing protein capable of demethylating tri- and dimethyl H3K27.

[1]The Wistar Institute, 3601 Spruce Street, Philadelphia, PA 19104, USA. [2]Institució Catalana de Recerca i Estudis Avançats and Centre de Regulació Genòmica, Barcelona, Spain. [3]Howard Hughes Medical Institute, Department of Biochemistry, New York University School of Medicine, New York, NY 10016, USA.

*To whom correspondence should be addressed. E-mail: ramin.shiekhattar@crg.es

We affinity-purified recombinant human UTX using a baculovirus expression system (Fig. 1A and fig. S1, A and B) and assessed its activity in demethylation assays. UTX has a specific demethylation activity toward tri- and dimethyl H3K27 without affecting methylation on H3K4, H3K9, H3K36, H3K79, and H4K20 (Fig. 1B and fig. S1C). Monomethylated H3K27 was not affected to the same extent when we used comparable concentrations of UTX that diminished di- and trimethylated species (Fig. 1B). The demethylation activity of UTX toward di- and trimethyl H3K27 was confirmed by mass spectrometric analysis of methylated H3K27 peptides corresponding to the H3 tail (fig. S2). Point mutations (H1146A and E1148A) in the catalytic JmjC-domain of UTX abrogated enzymatic activity, substantiating its role as an H3K27 demethylase (Fig. 1C and fig. S1B). Ectopic expression of wild-type UTX, but not its catalytic mutant, resulted in a global decrease in di- and trimethyl H3K27 levels (fig. S3A). Although the tetratricopeptide repeats (TPRs) present in UTX were not essential for the demethylation activity of UTX, the enzyme without TPRs displayed reduced activity (fig. S3, B and C). As is characteristic for the JmjC class of enzymes (*12*–*22*), demethylation activity by UTX required the addition of Fe(II) and ascorbate (Fig. 1D). However, similar to the case of JARID1d (a trimethyl H3K4 demethylase), we did not find a requirement for exogenously added α-ketoglutarate for UTX activity (Fig. 1D).

Recent genome-wide mapping of polycomb target genes revealed that polycomb group proteins and trimethyl H3K27 are enriched in *HOXA-D* loci (*23*), whose activity is required for embryonic development and when misregulated may lead to carcinogenesis. To assess the demethylation properties of UTX in vivo, we analyzed expression levels of *HOXA* and *HOXC* cluster genes using quantitative reverse transcription polymerase chain reaction (qRT-PCR) after the depletion of UTX with small interfering RNAs (siRNAs) (fig. S4, A and B). Immunostaining analysis using UTX antibodies revealed nuclear staining for endogenous UTX (fig. S5, A and B). Quantitative analysis of mRNA levels indicated that a number of genes in both *HOXA* and *HOXC* clusters are repressed after the knockdown of UTX (Fig. 1, E and F). Specifically, HOXA 6, 7, and 13 as well as HOXC4, 5, 6, 8, and 12 displayed greater than 50% repression after UTX depletion.

To demonstrate that UTX mediates such transcriptional effects on *HOX* genes directly, we examined UTX occupancy on the promoter as well as the body of the *HOXA13* and *HOXC4* genes with the use of a quantitative chromatin immunoprecipitation (qChIP) assay. We found a nearly 20-fold enrichment of UTX on the *HOXA13* and *HOXC4* promoters, but only two- to five-fold enrichment on the coding region (Fig. 2, A to D). The depletion of UTX (ChIP analysis revealed about a 50% decrease of UTX occupancy at the *HOXA13* and *HOXC4* promoters; Fig. 2, I and J) resulted in increased levels of di- and trimethyl H3K27 at the promoters of *HOXA13* and *HOXC4* but not at the 3′ end of these genes, consistent with the role of UTX as a H3K27 demethylase at the promoter of these genes (Fig. 2, E to H). We also examined the recruitment of PRC1 to *HOXA13* and *HOXC4*, which is targeted to methylated H3K27 sites and possess H2A ubiquitin ligase activity (*24*, *25*). The depletion of UTX leads to an increased occupancy of the Ring finger components of the PRC1 complex (Bmi1 and Ring1A proteins) and a concomitant enhancement of monoubiquitinated H2A at *HOXA13* and *HOXC4* (Fig. 2, I and J). In contrast, there was no change in the levels of histone H3 or in other histone modifications examined after UTX knockdown (Fig. 2, I and J). Taken together, these results indicate that the demethylation of

H3K27 by UTX regulates the occupancy of the PRC1 complex at UTX target genes, leading to the modulation of the transcriptional output.

To assess the recently described UTX interaction with mixed-lineage leukemia (MLL)–containing complexes (*26*, *27*), we developed a human embryonic kidney (HEK) 293–derived stable cell line expressing Flag-UTX. Analysis of Flag-UTX affinity eluate by mass spectrometry revealed the association of UTX with multiple components of MLL2/3-containig complexes (Fig. 3A and table S1). The Flag-UTX complex displayed H3K27 demethylase as well as H3K4 methyltransferase activities (Fig. 3, B to D). Analysis of Flag-UTX affinity eluate by gel filtration further demonstrated the association of Flag-UTX with a ~2-megadalton (MD) MLL2/3-containing complex (Fig. 3E). To extend this analysis, we developed stable cell lines expressing Flag–WD repeat domain 5 (WDR5), a core component of the MLL-containing complexes. WDR5-containing complexes specifically associate with UTX (fig. S6, A and B). Moreover, the UTX/WDR5-containing complexes display H3K4 methyltransferase activity toward recombinant nucleosomes (fig. S6C). Analysis of WDR5-containing complexes by Superose 6 gel filtration revealed that a fraction of WDR5 associates with UTX in a complex of ~2 MD displaying H3K4 methyltransfarse activity (fig. S6D).

These observations prompted us to investigate the interplay between the MLL complex, H3K4 methylation, and the UTX protein at the promoter of the *HOXA13* and *HOXC4* genes. The depletion of UTX did not have a significant effect on promoter occupancy of components of either PRC2 or the MLL2/3 complex as measured by qChIP, nor was there any change in the di- or trimethyl H3K4 levels (fig. S7, A and B). These results indicate that although UTX is a component of MLL2/3-containing complexes, the depletion of UTX and a concomitant increase in H3K27 methylation do not trigger changes in either the occupancy of the components of the MLL2/3 complexes or the methylation status of H3K4 at the *HOXA13* and *HOXC4* promoters.

To assess whether UTX plays a role during cellular differentiation, we used the human pluripotent embryonal carcinoma cell line NT2/D1 that differentiates into neural lineages upon treatment with retinoic acid (RA). We examined the occupancy of UTX at genes known to be activated during differentiation after RA treatment. UTX is present at *HOXA1-3* and *HOXB1-3* promoters, and the treatment of NT2/D1 cells with RA results in increased occupancy of UTX at these genes (Fig. 4, A to D, and fig. S8, A and E). The increase in UTX occupancy at these *HOX* genes was paralleled with a decrease in both PRC2 and trimethyl H3K27, leading to the activation of transcription (Fig. 4, E to L, and fig. S8, B, C, F, G, and I to N). Such RA-induced transcriptional activation is accompanied by increased trimethylation of H3K4 and the recruitment of ASH2L, a critical component of the MLL complex (Fig. 4, M to T, and fig. S8, D and H). In addition, whereas the recruitment of ASH2L and the trimethylation of H3K4 occur at 18 hours, the increased occupancy of UTX and the demethylation of H3K27 appear at a later 24-hour time point (Fig. 4). This suggests an ordered recruitment of an H3K4 methyltransferase and the H3K27 demethylase after RA-induced activation. Taken together, these results indicate that components of the MLL complex and UTX could be recruited to RA-responsive genes after RA stimulation to promote the methylation of H3K4, demethylation of H3K27, and a consequent activation of transcription.

In contrast to *HOXA1* to *A3* and *HOXB1* to *B3*, we did not find either UTX or trimethyl H3K27 at the promoter of the *OCT4* gene, a regulator of pluripotency, during the RA-induced repression of OCT4 transcription (fig. S9, A, C, and E). Moreover, whereas UTX is present at the *HOXA13* promoter, which is transcriptionally active in undifferentiated cells, RA treatment does not result in further recruitment of UTX or increased trimethyl H3K27 during RA-induced transcriptional repression (fig. S9, B, D, and F). These results indicate that the repression of *OCT4* and *HOXA13* upon RA treatment in NT2/D1 cells is probably regulated through a distinct mechanism other than that mediated by H3K27 methylation.

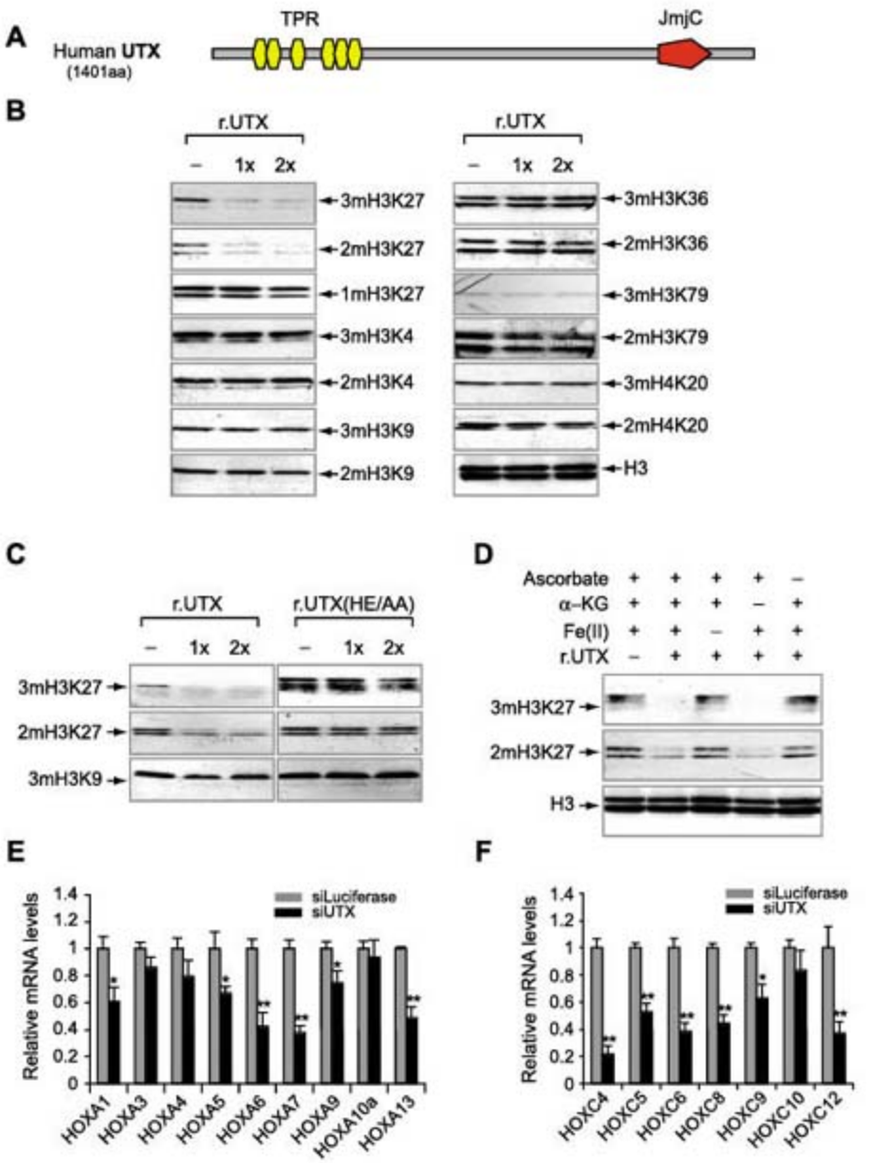


Fig. 1. UTX demethylates tri- and dimethyl H3K27 and regulates expression of several *HOX* genes. **(A)** Diagrammatic representation of UTX containing six TPRs and one JmjC. **(B)** Demethylation assay using recombinant (r.) UTX. **(C)** Comparison of demethylase activities of recombinant UTX and its catalytic mutant [r.UTX (HE/AA)]. **(D)** Demethylation activities of recombinant UTX (~800 ng) in the presence (+) or absence (–) of cofactors. α-KG, α-ketoglutarate. For data in (B) to (D), histones were mixed with recombinant proteins, and reaction mixtures were subjected to SDS–polyacrylamide gel electrophoresis, followed by Western blot analysis using antibodies to various methyl marks. 1x, ~400 ng of r.UTX or its mutant; 3m, trimethyl; 2m, dimethyl; 1m, monomethyl. **(E** and **F)** Analysis of mRNA levels of *HOXA* (E) and *HOXC* (F) clusters by qRT-PCR after treatment of HEK293 cells with siRNAs (siLuciferase or siUTX). Data are presented as the mean ± SEM (error bars) ($n = 3$). *($P < 0.05$) and **($P < 0.01$) indicate statistically significant changes (student's t test).

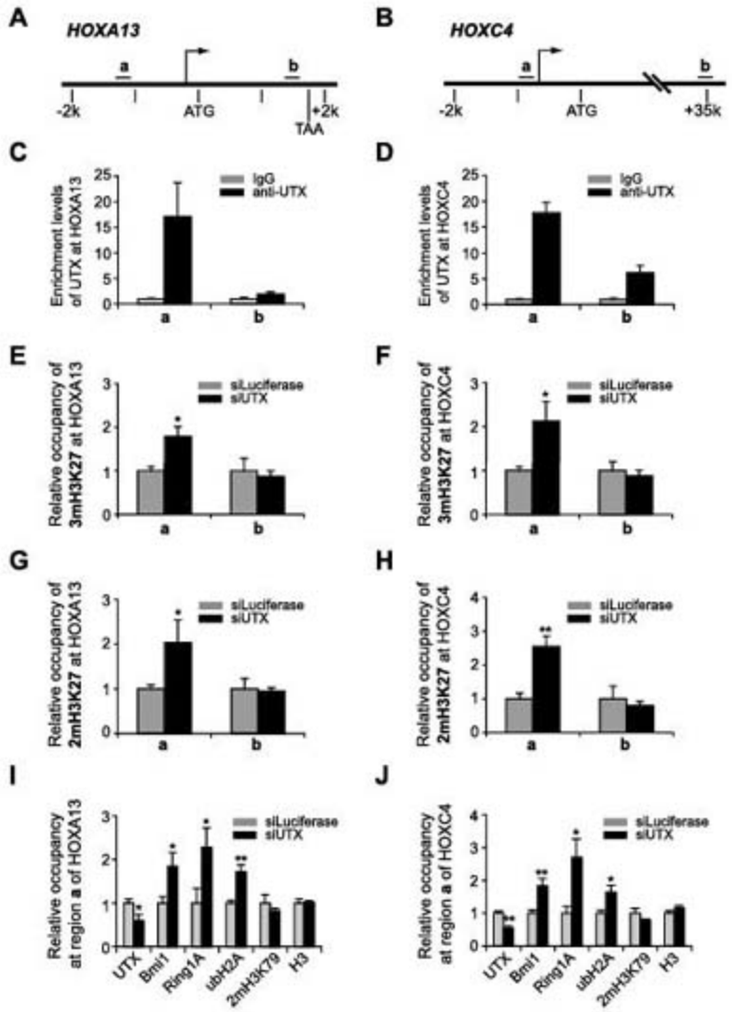


Fig. 2. UTX regulates the recruitment of PRC1 and ubiquitination of H2A. (**A** and **B**) Diagrammatic representation of the promoter and body of the *HOXA13* (A) and *HOXC4* (B) genes. a and b indicate regions amplified by PCR. (**C** and **D**) Analysis of UTX levels at *HOXA13* (C) and *HOXC4* (D) genes by a qChIP assay. IgG, immunoglobulin G. (**E** and **F**) Analysis of trimethyl H3K27 levels at *HOXA13* (E) and *HOXC4* (F) genes by qChIP. (**G** and **H**) Analysis of dimethyl H3K27 levels at *HOXA13* (G) and *HOXC4* (H) genes by qChIP. (**I** and **J**) Analysis of promoter occupancy of UTX, PRC1 subunits (Bmi1 and Ring1A), and ubiquitinated H2A (ubH2A) at region a of *HOXA13* (I) and *HOXC4* (J) genes. The relative occupancy represents the fold change in percent input over the control (*28*). The representative values of percent input set as 1 for *HOXA13* and *HOXC4* are as follows: UTX, 0.12/0.17; 3mH3K27, 1.2/0.19; 2mH3K27, 0.04/0.03; Bmi1, 0.01/0.02; RING1A, 0.01/0.03; ubH2A, 0.01/0.01; 2mH3K79, 0.43/2.24; and H3, 0.8/0.2. For the ChIP data in (E) to (J), HEK293 cells were treated with siRNAs (siLuciferase or siUTX). Data are presented as the mean ± SEM (error bars) ($n \geq 3$). *($P < 0.05$) and **($P < 0.01$) indicate statistically significant changes (student's t test).

In this work, we show that UTX, a component of MLL2/3 complexes, is a JmjC-domain-containing histone demethylase with the ability to specifically demethylate di- and trimethylated H3K27. Our in vivo studies demonstrated a critical role for UTX in the regulation of transcription, as well as levels of H3K27 methylation at *HOX* gene clusters. We provide evidence that UTX regulates transcription of *HOXA* and *HOXC* genes by modulating the recruitment of PRC1 and the monoubiquitination of histone H2A. Our results suggest an ordered cycle of H3K4 trimethylation brought about by the components of the MLL complex, followed by a wave of demethylation of H3K27 mediated by UTX during RA-induced transcriptional activation of *HOXA* and *HOXB* cluster genes in NT2/D1 cells. Taken together, these findings reveal that similar to other histone methylation sites, the methylation of H3K27 is a reversible process regulated by cellular signaling events.

References and Notes

1. R. J. Sims III, K. Nishioka, D. Reinberg, *Trends Genet.* **19**, 629 (2003).
2. C. Martin, Y. Zhang, *Nat. Rev. Mol. Cell Biol.* **6**, 838 (2005).
3. T. Kouzarides, *Curr. Opin. Genet. Dev.* **12**, 198 (2002).
4. A. Sparmann, M. van Lohuizen, *Nat. Rev. Cancer* **6**, 846 (2006).
5. J. P. Etchegaray *et al.*, *J. Biol. Chem.* **281**, 21209 (2006).
6. K. Plath *et al.*, *Science* **300**, 131 (2003).
7. A. Kuzmichev, K. Nishioka, H. Erdjument-Bromage, P. Tempst, D. Reinberg, *Genes Dev.* **16**, 2893 (2002).
8. R. Cao *et al.*, *Science* **298**, 1039 (2002).
9. B. Czermin *et al.*, *Cell* **111**, 185 (2002).
10. A. Kirmizis *et al.*, *Genes Dev.* **18**, 1592 (2004).
11. A. Barski *et al.*, *Cell* **129**, 823 (2007).
12. S. Iwase *et al.*, *Cell* **128**, 1077 (2007).
13. Y. Tsukada *et al.*, *Nature* **439**, 811 (2006).
14. K. Yamane *et al.*, *Cell* **125**, 483 (2006).
15. J. R. Whetstine *et al.*, *Cell* **125**, 467 (2006).

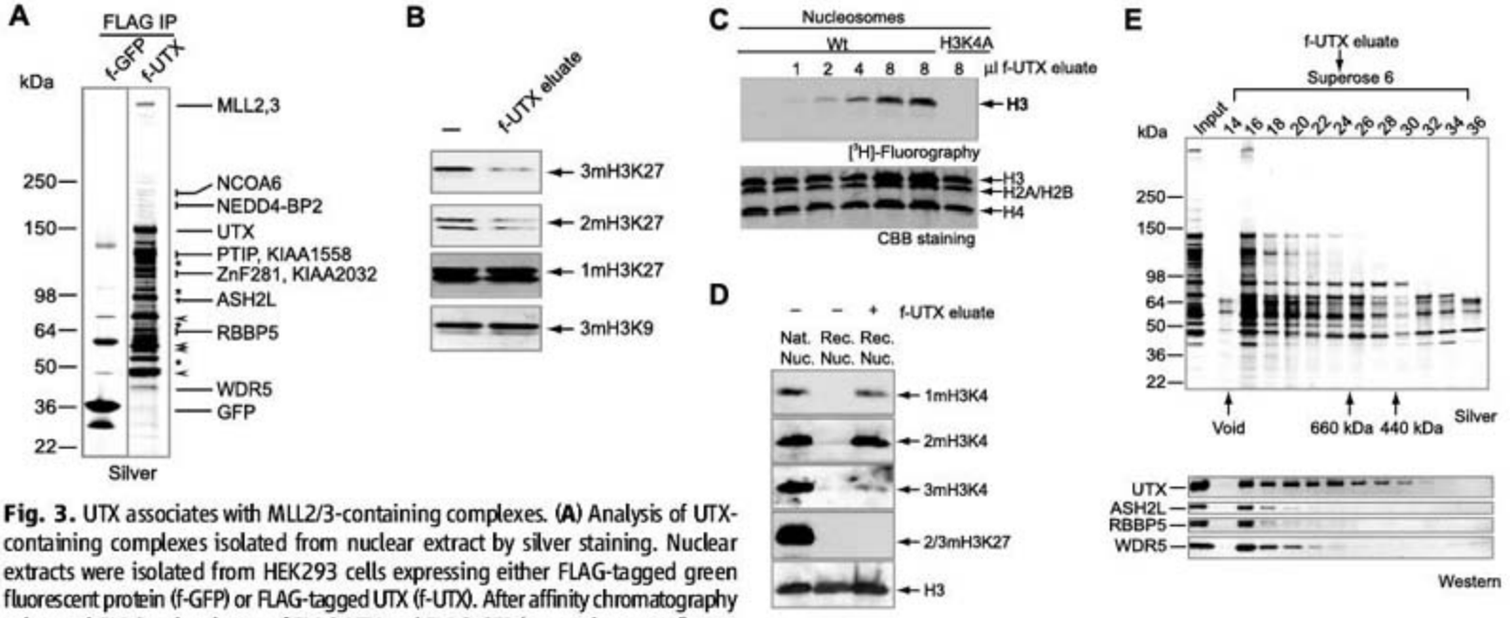


Fig. 3. UTX associates with MLL2/3-containing complexes. (**A**) Analysis of UTX-containing complexes isolated from nuclear extract by silver staining. Nuclear extracts were isolated from HEK293 cells expressing either FLAG-tagged green fluorescent protein (f-GFP) or FLAG-tagged UTX (f-UTX). After affinity chromatography using anti-FLAG resin, eluates of FLAG-UTX and FLAG-GFP (a negative control) were analyzed. GenBank accession numbers for UTX-associated proteins and numbers of peptides identified by mass spectrometric analysis are described in table S1. MLL2 was described as MLL4 in a recent report (*26*). The asterisks and arrowheads denote breakdowns and nonspecific polypeptides, respectively. IP, immunoprecipitation. (**B**) Demethylation assay using the FLAG-UTX complex. Histones were used as a substrate. (**C**) Histone lysine methyltransferase (HKMT) assay using the FLAG-UTX complex. Reconstituted nucleosomes containing wild-type (Wt) recombinant H3 or mutant H3 ($Lys^4 \rightarrow Ala^4$) were used as substrates. CBB, Coomassie brilliant blue. (**D**) Western blot analysis of an HKMT assay performed as in (C), using antibodies against various methyl marks. Native (Nat) nucleosomes (Nuc) were used as a positive control for Western blot analysis. Rec, reconstituted. (**E**) Silver staining and Western blot analysis of the UTX complex fractionated by Superose 6 gel filtration.

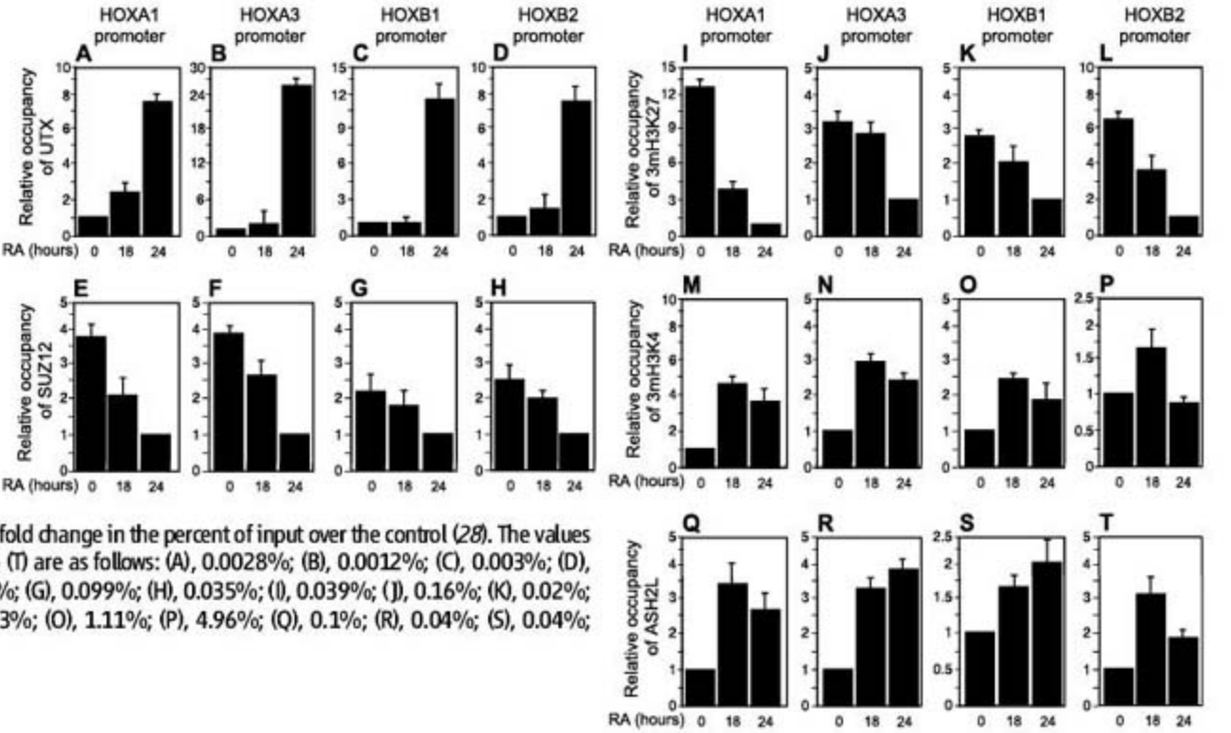


Fig. 4. RA treatment results in recruitment of the UTX/MLL complex, concomitant with decreased levels of trimethyl H3K27 and increased levels of trimethyl H3K4. (**A** to **T**) UTX occupancy (A to D), SUZ12 occupancy (E to H), trimethyl H3K27 levels (I to L), trimethyl H3K4 levels (M to P), and ASH2L occupancy (Q to T) at *HOXA1*, *HOXA3*, *HOXB1*, and *HOXB2* genes were analyzed by a qChIP assay. Data are presented as the mean ± SEM (error bars) ($n \geq 3$ except in the case of ASH2L, where $n = 2$). The relative occupancy represents the fold change in the percent of input over the control (*28*). The values of percent input set as 1 in (A) to (T) are as follows: (A), 0.0028%; (B), 0.0012%; (C), 0.003%; (D), 0.004%; (E), 0.046%; (F), 0.031%; (G), 0.099%; (H), 0.035%; (I), 0.039%; (J), 0.16%; (K), 0.02%; (L), 0.022%; (M), 2.47%; (N), 6.3%; (O), 1.11%; (P), 4.96%; (Q), 0.1%; (R), 0.04%; (S), 0.04%; and (T), 0.07%.

16. P. A. Cloos *et al.*, *Nature* **442**, 307 (2006).
17. R. J. Klose *et al.*, *Nature* **442**, 312 (2006).
18. B. D. Fodor *et al.*, *Genes Dev.* **20**, 1557 (2006).
19. M. G. Lee, J. Norman, A. Shilatifard, R. Shiekhattar, *Cell* **128**, 877 (2007).
20. R. J. Klose *et al.*, *Cell* **128**, 889 (2007).
21. J. Christensen *et al.*, *Cell* **128**, 1063 (2007).
22. J. C. Eissenberg *et al.*, *Nat. Struct. Mol. Biol.* **14**, 344 (2007).
23. A. P. Bracken, N. Dietrich, D. Pasini, K. H. Hansen, K. Helin, *Genes Dev.* **20**, 1123 (2006).
24. R. Cao, Y. Tsukada, Y. Zhang, *Mol. Cell* **20**, 845 (2005).
25. H. Wang *et al.*, *Nature* **431**, 873 (2004).
26. Y. W. Cho *et al.*, *J. Biol. Chem.* **282**, 20395 (2007).
27. I. Issaeva *et al.*, *Mol. Cell. Biol.* **27**, 1889 (2007).
28. Materials and methods are available as supporting material on *Science* Online.

29. We thank A. Shilatifard and T. Jenuwein for their antibodies; Kazusa DNA Research Institute Foundation for UTX cDNA; M. Hart, T. Beer, K. Speicher, and D. Speicher for help with mass spectrometry; G. Harris for figure preparation; and H. Hoff and D. Schultz for protein expression. L.D.C. was supported by a grant from the Spanish–Ministerio de Educación y Cultura and R.S. was supported by a grant from NIH (RO1CA090758) and an intramural fund from Centre de Regulació Genòmica.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1149042/DC1
Materials and Methods
Figs. S1 to S9
Table S1
References

5 June 2007; accepted 20 August 2007
Published online 30 August 2007;
10.1126/science.1149042
Include this information when citing this paper.

Permuted tRNA Genes Expressed via a Circular RNA Intermediate in *Cyanidioschyzon merolae*

Akiko Soma,[1] Akinori Onodera,[1] Junichi Sugahara,[2,3] Akio Kanai,[2,3,4] Nozomu Yachie,[2,3] Masaru Tomita,[2,3,4] Fujio Kawamura,[1,5] Yasuhiko Sekine[1,5]*

A computational analysis of the nuclear genome of a red alga, *Cyanidioschyzon merolae*, identified 11 transfer RNA (tRNA) genes in which the 3′ half of the tRNA lies upstream of the 5′ half in the genome. We verified that these genes are expressed and produce mature tRNAs that are aminoacylated. Analysis of tRNA-processing intermediates for these genes indicates an unusual processing pathway in which the termini of the tRNA precursor are ligated, resulting in formation of a characteristic circular RNA intermediate that is then processed at the acceptor stem to generate the correct termini.

Cyanidioschyzon merolae is an ultrasmall unicellular red alga that inhabits an extreme environment (*1*). The complete sequence of the nuclear genome of *C. merolae* recently became available (*2*). Genome-wide analyses (*1*, *2*) and a molecular phylogenetic analysis (*3*) have demonstrated that this organism is likely to represent one of the most ancestral forms of eukaryote. A search for tRNA genes from the *C. merolae* nuclear genome, using the tRNAscan-SE program (*4*), predicted only 30 tRNA genes encoding 30 species of anticodon, a number that is insufficient to decode all 61 codons (*2*). This prominent paucity of tRNA genes prompted us to search for undiscovered tRNA genes that may elucidate the evolution of tRNAs in early eukaryotes.

To search for *C. merolae* nuclear tRNA genes, we used SPLITS and SPLITSX, new programs that can detect cis-spliced tRNAs containing introns in various positions and trans-spliced tRNAs that are joined at several positions (*5*–*7*). In addition to this analysis, we performed a BLAST search of tRNA genes with conserved sequences in the TΨC arm or the anticodon arm, followed by manual inspection of the results.

[1]Department of Life Science, College of Science, Rikkyo (St. Paul's) University, Toshima, Tokyo 171-8501, Japan. [2]Institute for Advanced Biosciences, Keio University, Tsuruoka, 997-0017, Japan. [3]Systems Biology Program, Graduate School of Media and Governance, Keio University, Fujisawa, 252-8520, Japan. [4]Faculty of Environment and Information Studies, Keio University, Fujisawa, 252-8520, Japan. [5]Research Information Center for Extremophile, Rikkyo (St. Paul's) University, Toshima, Tokyo 171-8501, Japan.

*To whom correspondence should be addressed. E-mail: ysekine@rikkyo.ac.jp

The most important finding was that 11 genes have a novel gene organization in which the 3′ half of the tRNA sequence lies upstream of the 5′ half in the genome (Fig. 1A and fig. S1). Such a gene arrangement is accomplished by circular gene permutation (*8*), and we therefore termed these genes permuted tRNA genes. As shown in fig. S1, a TATA-like sequence is found upstream of the 3′ half in most of the genes. This sequence is also conserved in nonpermuted tRNA genes of the *C. merolae* nuclear genome. Thus, instead of the intragenic bipartite promoter consisting of an A box and a B box, which are conserved sequences in the D arm and TΨC arm (*9*), the upstream TATA-like sequence may play a central role in initiation of transcription in *C. merolae*. The genomic sequence encoding the intervening sequence between the 3′ and 5′ halves varies from 7 to 74 base pairs (bp). Downstream of the 5′ half, a T stretch that corresponds to a termination signal for RNA polymerase III (pol III) is found (*10*). These observations suggest that the pair of putative tRNA halves is transcribed as a linear RNA. As shown in Fig. 1B and fig. S2, permuted tRNA genes can be classified into four types on the basis of the location of the junction between the 3′ end of the 5′ half and the 5′ end of the 3′ half in the inferred secondary structures of the tRNAs. The junctions are located at 20/21 (between position 20 and 21) in the D loop (I), 37/38 in the anticodon loop (II), 50/51 in the TΨC stem (III), and 59/60 in the TΨC loop (IV). We identified one, six, one, and three candidates

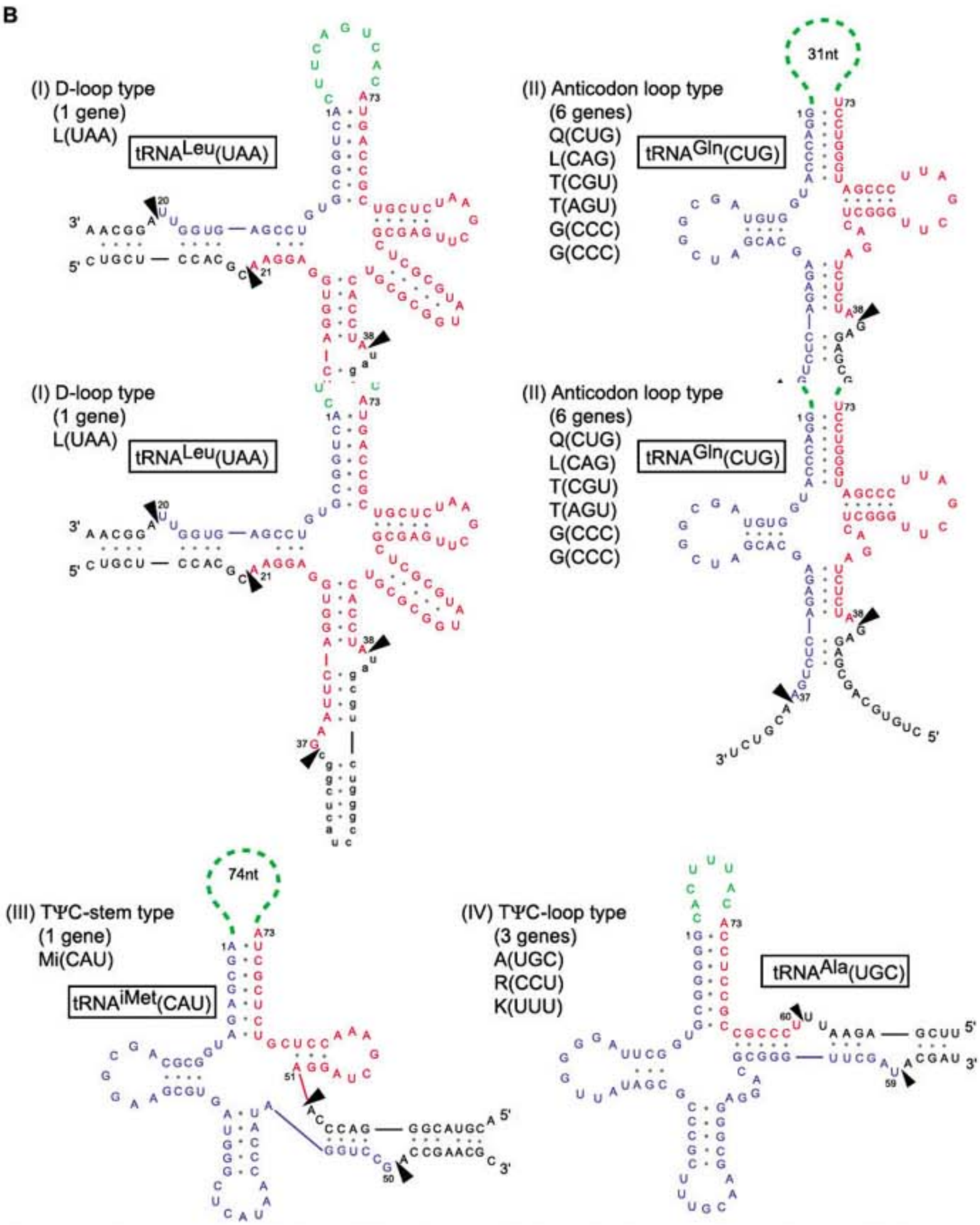


Fig. 1. Permuted tRNA genes in *C. merolae*. **(A)** Schematic representation of a permuted tRNA gene. **(B)** Inferred secondary structures for pre-tRNAs of four types of permuted tRNA genes. Arrowheads indicate the positions to be processed. The intron sequence is shown in lower case. The numbering of the tRNA positions is according to (*19*). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; G, Gly; K, Lys; L, Leu; M, Met; Q, Gln; R, Arg; and T, Thr.

for the type I to IV tRNAs, respectively. Notably, the sequences adjacent to those junctions in tRNA precursors (pre-tRNAs) potentially form bulge-helix-bulge (BHB) motifs, which were originally found around the intron-exon junctions of nuclear and archaeal tRNAs (*11*).

Northern blot analysis using *C. merolae* total RNA verified that permuted tRNA genes are indeed transcribed in the cell (fig. S3). Next, to examine whether these tRNAs are aminoacylated, we performed Northern blot analysis of acid-urea gels with use of total RNA prepared from *C. merolae* cells under acidic conditions. Putative aminoacylated forms, which migrate more slowly than the deacylated forms, were detected, showing that these tRNAs could be aminoacylated in vivo (Fig. 2, lanes 1 and 2, and fig. S4). An in vitro aminoacylation analysis showed that recombinant *C. merolae* methionyl-tRNA synthetase (MetRS) methionylates $tRNA^{iMet}$ in total RNA preparations (Fig. 2, lanes 3 to 5). An in vitro transcribed $tRNA^{iMet}$ with the 3′ terminal CCA sequence was methionylated, although with less efficiency (Fig. 2, lanes 6 to 9). Thus, tRNA molecules expressed from permuted tRNA genes are aminoacylated and are likely to participate in protein synthesis.

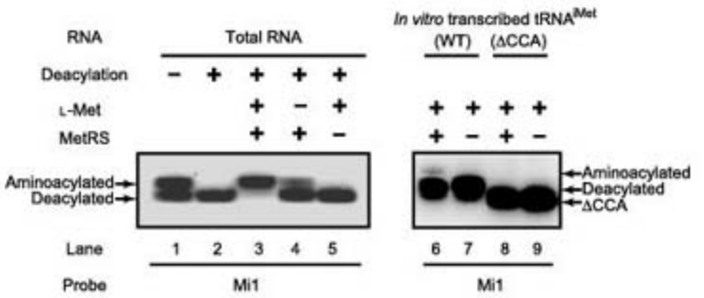


Fig. 2. Aminoacylation analysis of $tRNA^{iMet}$(CAU). Total *C. merolae* RNA prepared under acidic conditions was separated on an acid-urea gel directly (lane 1) or after deacylation (lane 2). In vitro methionylation reaction mixtures were loaded onto the gel (lanes 3 to 9). Deacylated total RNA (lanes 3 to 5) or in vitro transcribed $tRNA^{iMet}$ with [wild type (WT), lanes 6 and 7] or without (ΔCCA, lanes 8 and 9) the CCA sequence was used.

What is the processing mechanism of the pre-tRNA for these unusual tRNA genes? To clarify the processing pathway, we detected processing intermediates by reverse transcription polymerase chain reaction (RT-PCR) with two different sets of primers (Fig. 3A), followed by sequencing analysis of those RT-PCR products (Fig. 3B and fig. S5). Analysis of the $tRNA^{Gln}$(CUG) verified the sequences of the pre-$tRNA^{Gln}$ with a circularly permuted structure in which the leader sequence, the 3′ half, the intervening sequence, the 5′ half, and the trailer sequence were aligned in this order (Fig. 3B, lane 1, and fig. S5A). Interestingly, we also detected a circular RNA intermediate in which the leader and trailer sequences were removed and the resulting ends were ligated, while the intervening sequence was retained (Fig. 3B, lane 2, and fig. S5B). The existence of the circular RNA intermediate was confirmed by the generation of a PCR product representing two rounds of reverse transcription around the circular RNA (Fig. 3B, lane 2). The 3′-terminal CCA sequence is added posttranscriptionally in eukaryotes (*12*). To determine the terminal sequence of the mature $tRNA^{Gln}$, we performed RT-PCR with total RNA circularized by T4 RNA ligase. The sequence of the mature $tRNA^{Gln}$, in which the extra sequences are removed and the CCA sequence is added to the 3′ terminus of the acceptor stem, was verified (Fig. 3B, lane 3, and fig. S5C). As summarized in the model presented in Fig. 3C, maturation of the pre-tRNA probably starts with processing of the leader and trailer sequences, resulting in formation of the circular RNA intermediate. This processing step is most likely carried out by the tRNA-splicing machinery because the sequences adjoining the processing sites potentially form a BHB motif, which is the dominant recognition element for nuclear and archaeal tRNA-splicing endonucleases (*11*). The intervening sequence is then removed, possibly by ribonuclease (RNase) P and tRNase Z (*13*, *14*), followed by the CCA addition, to generate the correct termini. This model would be common to the permuted tRNA genes, because the circular RNA intermediate was detected for all 11 genes.

How could permuted tRNA genes have arisen? Permuted noncoding RNA (ncRNA) genes have been reported for *Trypanosoma*

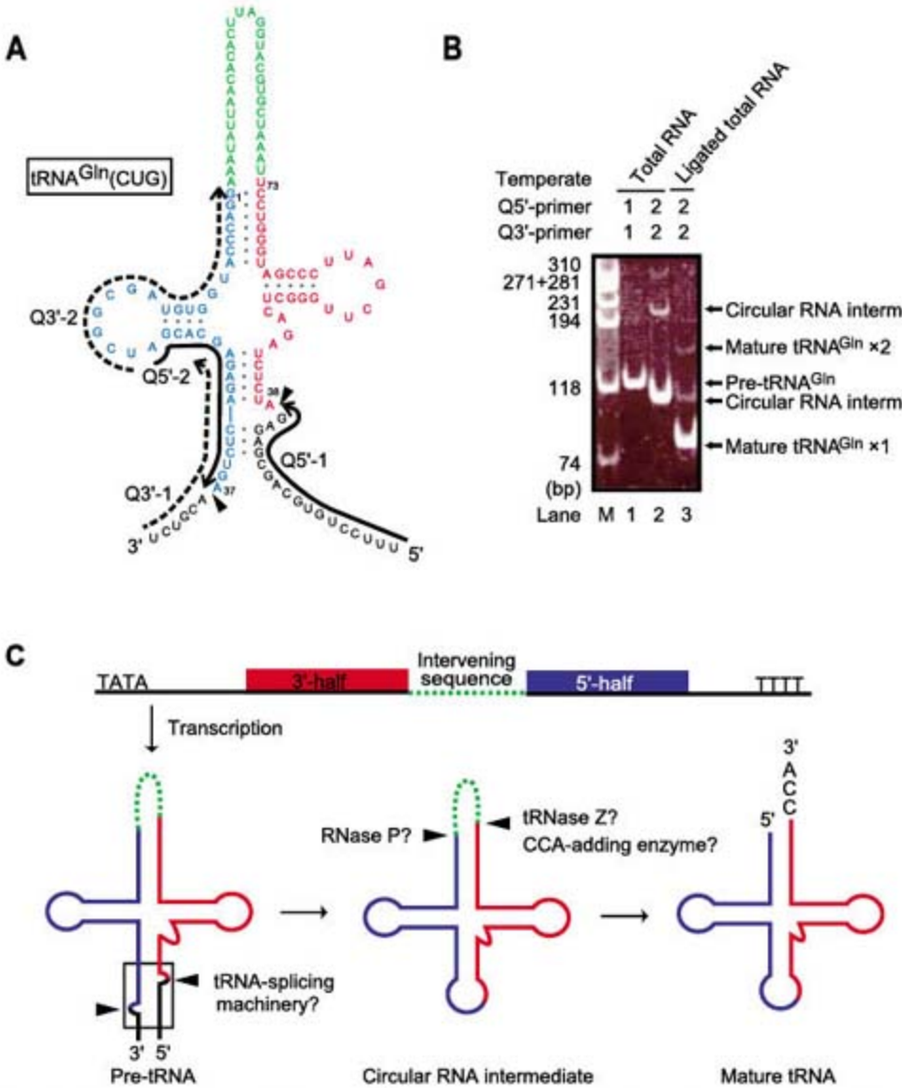


Fig. 3. RT-PCR amplification of $tRNA^{Gln}$(CUG) and a model for the maturation of the permuted pre-tRNAs. (**A**) 5′ and 3′ primers used for RT-PCR are indicated as solid and broken arrows, respectively. (**B**) PCR products amplified from cDNA of pre-$tRNA^{Gln}$ and from cDNA generated from one (×1) or two (×2) rounds of reverse transcription around a circular RNA intermediate or a circularized mature $tRNA^{Gln}$ are indicated. Lane M, DNA molecular weight markers (Φ×174 DNA-Hae III digest). (**C**) Maturation of the pre-tRNA starts with processing of a BHB motif (boxed) by the tRNA-splicing machinery, resulting in formation of a circular RNA intermediate. The intervening sequence is removed by RNase P and tRNase Z, then followed by the CCA addition.

mitochondrial small subunit (SSU) ribosomal RNA (rRNA) (*15*) and a bacterial transfer messenger RNA (tmRNA) (*16*) that function in a two-piece form, in contrast to the *C. merolae* tRNAs reported here that function in a one-piece form. The permuted rRNA and tmRNA genes are hypothesized to have arisen by a gene duplication that formed a tandem repeat, followed by the loss of the outer segment of each copy (*15*, *17*). Even if a circular permutation occurred in tRNA genes, most of the resulting permuted genes would not be retained in the genome because of the failure of expression or the loss of functional structure of the RNA. In *C. merolae*, however, permuted tRNA genes might have persisted in the genome because of the use of the upstream promoter by the transcription system and processing of the circularly permuted pre-tRNA into a canonical tRNA molecule by the tRNA-splicing machinery. Considering that *C. merolae* is an early rooted eukaryote and that the BHB motifs would play a pivotal role in the tRNA processing, it is possible that the permuted tRNA genes might have developed via a common process with the split-tRNA genes of *Nanoarchaeum equitans* (*18*). Further investigation should provide a hint about how to evaluate the evolution of tRNA genes in the early eukaryote.

References and Notes

1. T. Kuroiwa, *Bioessays* **20**, 344 (1998).
2. M. Matsuzaki *et al.*, *Nature* **428**, 653 (2004).
3. H. Nozaki *et al.*, *J. Mol. Evol.* **56**, 485 (2003).
4. T. M. Lowe, S. R. Eddy, *Nucleic Acids Res.* **25**, 955 (1997).
5. Materials and methods are available on *Science* Online.
6. J. Sugahara *et al.*, *In Silico Biol.* **6**, 411 (2006).
7. J. Sugahara, N. Yachie, K. Arakawa, M. Tomita, *RNA* **13**, 671 (2007).
8. T. Pan, O. C. Uhlenbeck, *Gene* **125**, 111 (1993).
9. G. Galli, H. Hofstetter, M. L. Birnstiel, *Nature* **294**, 626 (1981).
10. M. Hamada, A. L. Sakulich, S. B. Koduru, R. J. Maraia, *J. Biol. Chem.* **275**, 29076 (2000).
11. J. Abelson, C. R. Trotta, H. Li, *J. Biol. Chem.* **273**, 12685 (1998).
12. A. M. Weiner, *Curr. Biol.* **14**, R883 (2004).
13. S. Altman, L. Kirsebom, S. Talbot, *FASEB J.* **7**, 7 (1993).
14. H. Schurer, S. Schiffer, A. Marchfelder, M. Morl, *Biol. Chem.* **382**, 1147 (2001).
15. T. Y. Heinonen, M. N. Schnare, P. G. Young, M. W. Gray, *J. Biol. Chem.* **262**, 2879 (1987).
16. K. C. Keiler, L. Shapiro, K. P. Williams, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7778 (2000).
17. K. P. Williams, *Nucleic Acids Res.* **30**, 2025 (2002).
18. L. Randau *et al.*, *Nature* **433**, 537 (2005).
19. M. Sprinzl, K. S. Vassilenko, *Nucleic Acids Res.* **33**, D139 (2005).

20. Supported by Japan Society of the Promotion of Science (JSPS) Fellowships (7473 to A.S. and 1924 to N.Y.), by Grants-in-Aid for Creative Scientific Research (17GS0314 to Y.S. and 16GS0304 to F.K.) from JSPS and Ministry of Education, Culture, Sports, Science and Technology, respectively, by grants from the Yamagata Prefectural Government and Tsuruoka City in Japan (to M.T., A.K., J.S., and N.Y.), and by the Frontier project Adaptation and Evolution of Extremophile (to Y.S.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/450/DC1
Materials and Methods
Figs. S1 to S5
References and Notes

29 May 2007; accepted 31 August 2007
10.1126/science.1145718

Trojan Horse Strategy in *Agrobacterium* Transformation: Abusing MAPK Defense Signaling

Armin Djamei,[1]*† Andrea Pitzschke,[1]† Hirofumi Nakagami,[1]†‡ Iva Rajh,[1] Heribert Hirt[1,2]

Nuclear import of transfer DNA (T-DNA) is a central event in *Agrobacterium* transformation of plant cells and is thought to occur by the hijacking of certain host cell proteins. The T-DNA–associated virulence protein VirE2 mediates this process by binding to the nuclear import machinery via the host cell factor VIP1, whose role in plants has been so far unknown. Here we show that VIP1 is a transcription factor that is a direct target of the *Agrobacterium*-induced mitogen-activated protein kinase (MAPK) MPK3. Upon phosphorylation by MPK3, VIP1 relocalizes from the cytoplasm to the nucleus and regulates the expression of the *PR1* pathogenesis-related gene. MAPK-dependent phosphorylation of VIP1 is necessary for VIP1-mediated *Agrobacterium* T-DNA transfer, indicating that *Agrobacterium* abuses the MAPK-targeted VIP1 defense signaling pathway for nuclear delivery of the T-DNA complex as a Trojan horse.

Higher eukaryotes recognize microbes through pathogen-associated molecular patterns (PAMPs) and activate the innate immune response in animals and plants (*1*). The perception of PAMPs leads to rapid activation of host defense mechanisms, including the activation of mitogen-activated protein kinases (MAPKs), production of reactive oxygen species, and subsequent induction of defense-related genes. However, virulence factors of successful pathogens can inhibit PAMP-elicited basal defenses (*2*). In specific cases, plants have evolved resistance proteins specialized to detect these pathogen-derived virulence factors or their effects on host targets. As a consequence, a hypersensitive response (HR) occurs that includes localized cell death and the arrest of pathogen spread (*3*, *4*).

Agrobacterium transforms plants by transporting a single-stranded copy of the transfer DNA (T-DNA) from its tumor-inducing Ti plasmid into the host cell and integrating it into the host cell genome. The agrobacterial transformation process is mediated by Vir (virulence) proteins. The T-DNA strand that is exported into plant cells has the VirD2 protein covalently attached to its 5′ end. The VirE2 protein, which is translocated into plant cells independently of the T-DNA strand, associates with the T strand in the plant cell (*5*) before nuclear import mediated by cellular karyopherin α, which binds to VirD2. Nuclear import is further facilitated by the host protein VIP1, which functions as an adaptor (*6*), but whose cellular function is as yet unknown.

PAMPs activate a variety of plant MAPK cascades (*7*), which make a major contribution to the host defense responses. In *Arabidopsis thaliana*, PAMPs such as flagellin activate at least three MAPKs—MPK3, MPK4, and MPK6—resulting in altered expression of various stress-responsive genes (*8*–*10*). So far, the direct downstream targets of these plant MAPKs are largely unknown. In this study, we show that *Agrobacterium* triggers the activation of several MAPKs, including MPK3, and we identify VIP1 as a target of MPK3.

A yeast two-hybrid screen was performed in order to find interactors with MPK3. Clones carrying full-length cDNAs of VIP1 (VirE2-interacting protein At1g43700) (*11*) were repeatedly isolated. The VIP1-MPK3 interaction in yeast was confirmed by its ability to induce another reporter gene, *lacZ*, encoding for β-galactosidase (Fig. 1A). In order to test whether VIP1 also interacts with other MAPKs, targeted yeast two-hybrid interaction experiments were performed with VIP1 and representatives of all the *A. thaliana* MAPK subfamilies (MPK2, -3, -4, -5, -6, -7, -16, and -17). VIP1-MAPK interaction was specific for MPK3, because neither MPK6, the closest homolog of MPK3, nor any of the other MAPKs tested interacted with VIP1 (Fig. 1A).

To substantiate these results, we transiently expressed MPK3 and VIP1 in *Arabidopsis*

[1]Department of Plant Molecular Biology, Max F. Perutz Laboratories, University of Vienna, Dr.-Bohr-Gasse 9, 1030 Vienna, Austria. [2]URGV Plant Genomics Laboratory, 2 Rue Gaston Crémieux, 91057 Evry, France.

*Present address: Department of Organismic Interactions, Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Strasse, 35043 Marburg, Germany.
†These authors contributed equally to this work.
‡Present address: Plant Immunity Research Team, RIKEN Plant Science Center, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan.

protoplasts under control of the constitutive cauliflower mosaic virus 35*S* promoter as C-terminally tagged proteins with either hemagglutinin (HA) or Myc epitopes that allowed the immunoprecipitation and immunoblotting of the proteins. From protein extracts of protoplasts expressing MPK3-HA alone (negative control) or coexpressing MPK3-HA and VIP1-Myc, VIP1-Myc was immunoprecipitated using an antibody to Myc. MPK3-HA could be coimmunoprecipitated with VIP1-Myc (Fig. 1B). We also found coimmunoprecipitation of MPK3-HA with VIP1-phosphoderivatives VIP1_A-Myc and VIP1_D-Myc, as well as with its known activator MKK4-Myc (*8*), but not of MPK3-HA when expressed alone (Fig. 1B). These findings support the notion that VIP1 and MPK3 are true interaction partners.

VIP1 contains a single potential MAPK phosphorylation site, Ser^{79} (Fig. 1C). To test whether VIP1 can be targeted by MPK3, recombinant glutathione *S*-transferase (GST) fusion proteins of VIP1, VIP1_A (Ser^{79}→Ala^{79}, mimicking constitutively nonphosphorylated VIP1), and VIP1_D (Ser^{79}→Asp^{79}, mimicking constitutively phosphorylated VIP1) were produced. To obtain active MPK3, MPK3-HA was coexpressed in protoplasts with its upstream activator MKK4 and immunoprecipitated using an antibody to HA. When recombinant GST-VIP1 was incubated with immunoprecipitated MPK3-HA in the presence of ^{32}P-γ–labeled adenosine triphosphate, it became phosphorylated by MPK3, as did the artificial MAPK substrate myelin basic protein (MBP) but not GST (Fig. 1D). We also observed VIP1 phosphorylation by recombinant GST-MPK3 (fig. S1), excluding the possibility that VIP1 might be the substrate of a kinase that had been coimmunoprecipitated with MPK3-HA. Moreover, no signal was detected when the kinase assays were performed with recombinant VIP1 derivatives VIP1_A or VIP1_D that, due to mutation of Ser^{79}, lack a MAPK phosphorylation site (fig. S1). These results indicate that VIP1 is a substrate of MPK3 and that Ser^{79} in VIP1 corresponds to the phosphorylation site in VIP1.

It is thought that most MAPKs are located in the cytoplasm and relocalize to the nucleus after activation, where they phosphorylate transcription factors. In animals, the subcellular localization and thereby the activity of transcription factors can also be controlled through phosphorylation by a MAPK residing in the cytoplasm (*12*), but whether plant transcription factors can also be regulated in this way is so far unknown. Our results indicate that VIP1 can interact with and be phosphorylated by MPK3 (Fig. 1).

Whether VIP1 phosphorylation could affect its localization was addressed by in vivo confocal microscopy of transgenic *Arabidopsis* plants expressing C-terminally tagged VIP1-YFP fusion protein (YFP, yellow fluorescent protein). To this end, wild-type VIP1 and the mutated versions VIP1_A and VIP1_D were expressed under the control of an estradiol-inducible promoter. A minimum of six independent lines of each construct were analyzed. As shown in Fig. 2A, wild-type VIP1-YFP was detected in the cytoplasm and in the nucleus. VIP1_A-YFP showed a similar localization. In contrast, VIP1_D-YFP was predominantly nuclear. These results suggest that VIP1 can reside in both the nuclear and the cytoplasmic compartment and that its localization is regulated by the phosphorylation state of Ser^{79}. The reason why VIP1_A-YFP is not exclusively extranuclear and VIP1_D is not exclusively intranuclear might be due to homomultimerization with endogenous VIP1, because VIP1 has been shown to form homomultimers (*13*).

To investigate whether MPK3 activation could affect the subcellular localization of VIP1, we treated estradiol-induced VIP1-YFP plants with flg22 peptide corresponding to the highly conserved flagellin sequence of pathogenic bacteria (*8*). Plants recognize flg22 and initiate a signaling cascade that results in the activation of MPK3 within 5 min (*9*). Upon treatment with flg22, a strong decrease in the cytoplasmic and a concomitant increase in the nuclear pool of VIP1-YFP was observed (Fig. 2B), whereas VIP1_A-YFP showed no such relocalization (fig. S2). These results support the notion that MPK3 activity regulates the subcellular localization of VIP1 via the phosphorylation of Ser^{79}. Previous studies on VIP1 focused on its role during the transformation of plant cells by agrobacteria (*6*). Assuming that MPK3 is regulating VIP1 localization by phosphorylation, we then wondered whether MPK3 actually gets activated in plants by agrobacteria, which, unlike most pathogenic bacteria, have a distinct flagellin that is not recognized by the plant flagellin receptor FLS2 that is upstream of MPK3 and MPK6 (*8*). By in vitro kinase assays using MBP as a substrate, we analyzed the activity of MPK3 in 12-day-old *Arabidopsis* wild-type seedlings at 2, 5, and 10 min after treatment with agrobacteria. We also tested two other PAMP-activated MAPKs, MPK4 and MPK6. As compared to mock-treated seedlings, the activities of MPK3, MPK4, and MPK6 strongly increased upon incubation with the agrobacteria (Fig. 2D). Activation of these MAPKs could already be detected 5 min after

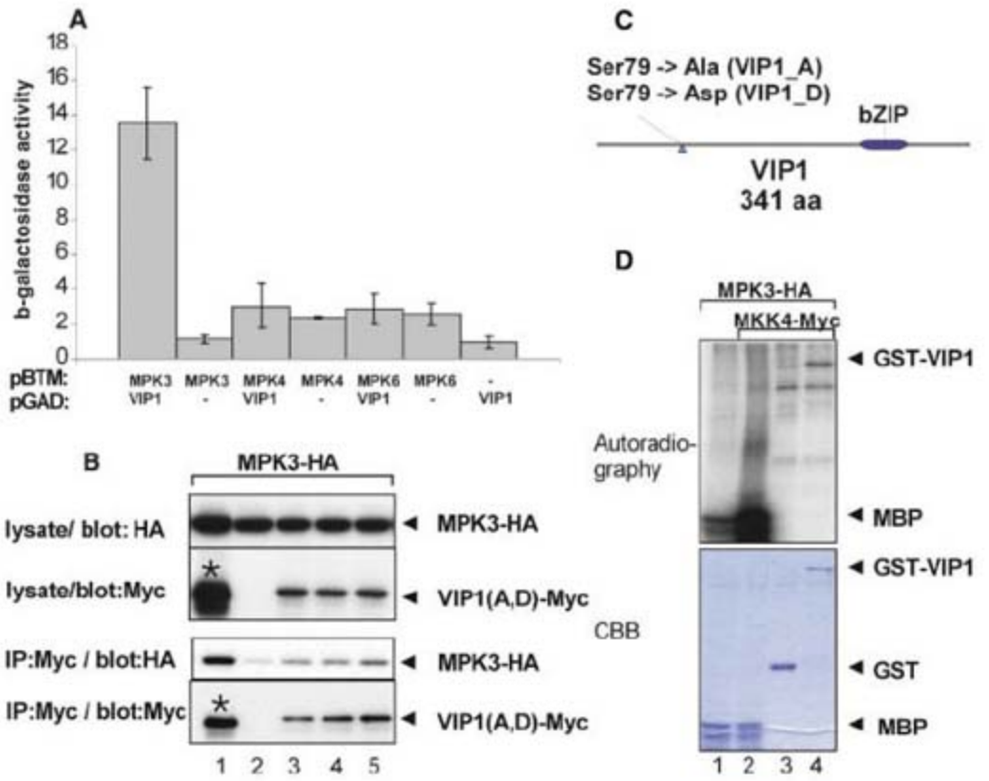


Fig. 1. VIP1 interacts with and is phosphorylated by MPK3. **(A)** Yeast two-hybrid analysis. The interaction of VIP1 (fused to activation domain, in pGAD) and MPK3, -4, and -6 (fused to binding domain, in pBTM) and the respective empty vector controls (–) as quantified by β-galactosidase activity assay is shown (n = 9 independent yeast cotransformants; error bars indicate SD). **(B)** Coimmunoprecipitation. Protoplasts were transfected with MPK3-HA alone (lane 2) or in combination with Myc-tagged VIP1 (lane 3) or its phosphoderivatives (VIP1_A, lane 4; VIP1_D, lane 5). Protoplast lysates were subjected to immunoprecipitation with rabbit antibody to Myc. Crude lysates (upper panel) and immunoprecipitated proteins (lower panel) were detected with mouse antibodies to HA and Myc. Cotransfection of MPK3-HA and its known interactor MKK4 (*, Myc-tagged) served as a positive control (lane 1). **(C)** Schematic diagram of VIP1 protein. The MAPK phosphorylation site, Ser^{79}, and bZIP domain are indicated. **(D)** In vitro kinase assay. MPK3 (lane 1) or activated MPK3 (lanes 2 to 4), which had been immunoprecipitated from protoplasts expressing MPK3-HA alone or in combination with its activator MKK4-Myc, respectively, were incubated with the following substrates: MBP (lanes 1 and 2, positive control), recombinant proteins GST (lane 3, negative control), or GST-VIP1 (lane 4). Autoradiography (upper panel) and Coomassie blue-stained SDS–polyacrylamide gel electrophoresis (CBB, lower panel) are shown.

treatment and further increased over time in the case of MPK3 and MPK6. These data show that *A. thaliana* recognizes agrobacteria and that this recognition triggers the activation of multiple MAPKs, including MPK3.

To investigate whether *Agrobacterium*-induced activation of MPK3 correlates with a relocalization of VIP1, estradiol-induced leaves of VIP1-YFP plants were treated with *Agrobacterium tumefaciens* and analyzed by confocal microscopy. Within 5 min of bacterial contact, wild-type VIP1-YFP accumulation was observed in leaf nuclei (Fig. 2C). Treatment with flg22 and agrobacteria triggered the aggregation of some VIP1-YFP as well as VIP1_A-YFP in vesicle-like structures (Fig. 2, B and C, and fig. S2).

Because VIP1 rapidly relocalizes to the nucleus upon contact with agrobacteria or the flg22 peptide, we hypothesized that VIP1 plays a more general role in host defense response. One typical response of the pathogen-induced MAPK pathway is expression of the pathogenesis-related *PR1* gene (*8*). We therefore investigated the potential effect of enhanced nuclear VIP1 levels on *PR1* expression in protoplasts transfected with a PR1 promoter–driven GUS construct (PR1::GUS) and VIP1-HA or PR1::GUS alone, respectively. Compared to protoplasts transfected with PR1::GUS only, GUS activity in protein extracts from protoplasts cotransfected with VIP1-HA and PR1::GUS was significantly enhanced (Fig. 3A). In order to verify that the nuclear localization of VIP1 is essential for its function in biotic stress gene modulation, we tested the effect of a membrane-targeted myristoylated/palmitoylated Myr-VIP1-YFP fusion protein on PR1 promoter–driven GUS expression in protoplasts. In contrast to VIP1-YFP, Myr-VIP1-YFP failed to induce PR1::GUS expression, confirming the functional relevance of the subcellular localization of VIP1 (Fig. 3A).

Because we observed *Agrobacterium*-triggered phosphorylation and nuclear translocation of VIP1, we then investigated whether the nuclear import of the VirE2/T-DNA complex (*11*) also relies on the phosphorylation of VIP1. To this end, *Arabidopsis* root cell

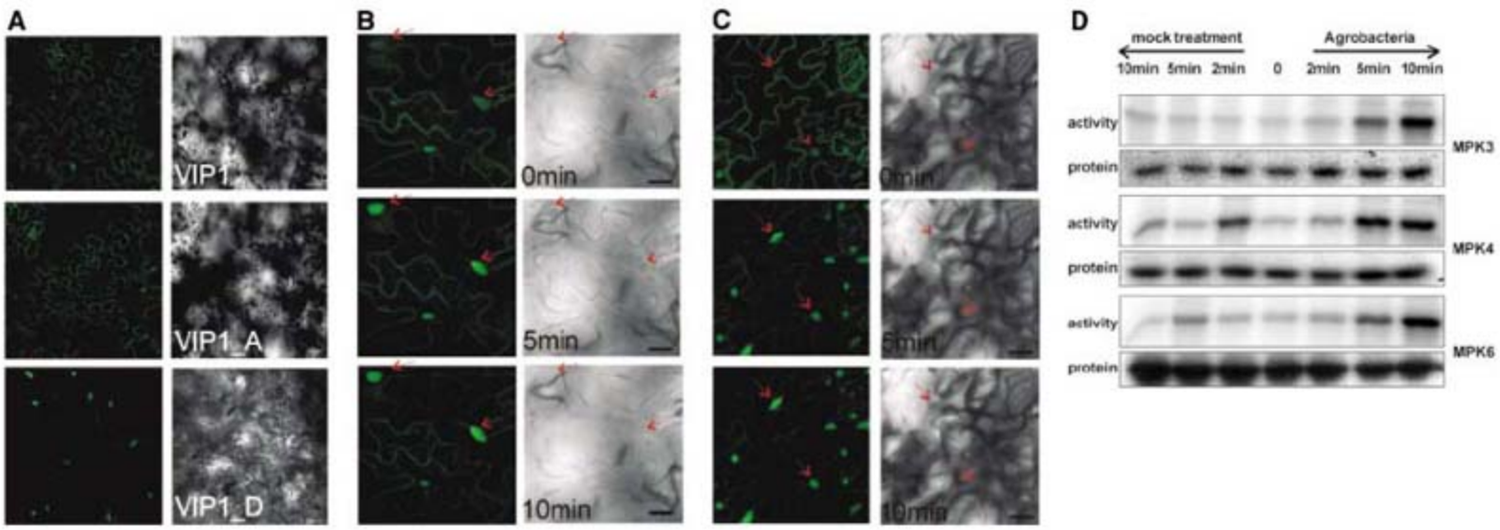


Fig. 2. Nuclear translocation of VIP1 is regulated by phosphorylation through stress-induced MPK3. (**A** to **C**) Confocal images of leaves from 5-week-old stable transgenic *A. thaliana* plants with estradiol-induced (40 μM for 12 hours) VIP1-YFP, VIP1_A-YFP, or VIP1_D-YFP expression. Left columns, YFP fluorescence; right columns, bright-field image. (B) VIP1 nuclear relocalization upon application of flagellin (1 μM) and (C) of an agrobacterial suspension. Scale bars, 20 μM. (**D**) *Agrobacterium*-induced MAPK activation. *A. thaliana* seedlings were treated with an agrobacterial suspension and harvested at the time points indicated. MAPKs were immunoprecipitated with specific antibodies, and their activity was monitored by in vitro kinase assays with the substrate MBP. The presence of equal amounts of the MAPK protein in each protein extract was confirmed by immunoblotting.

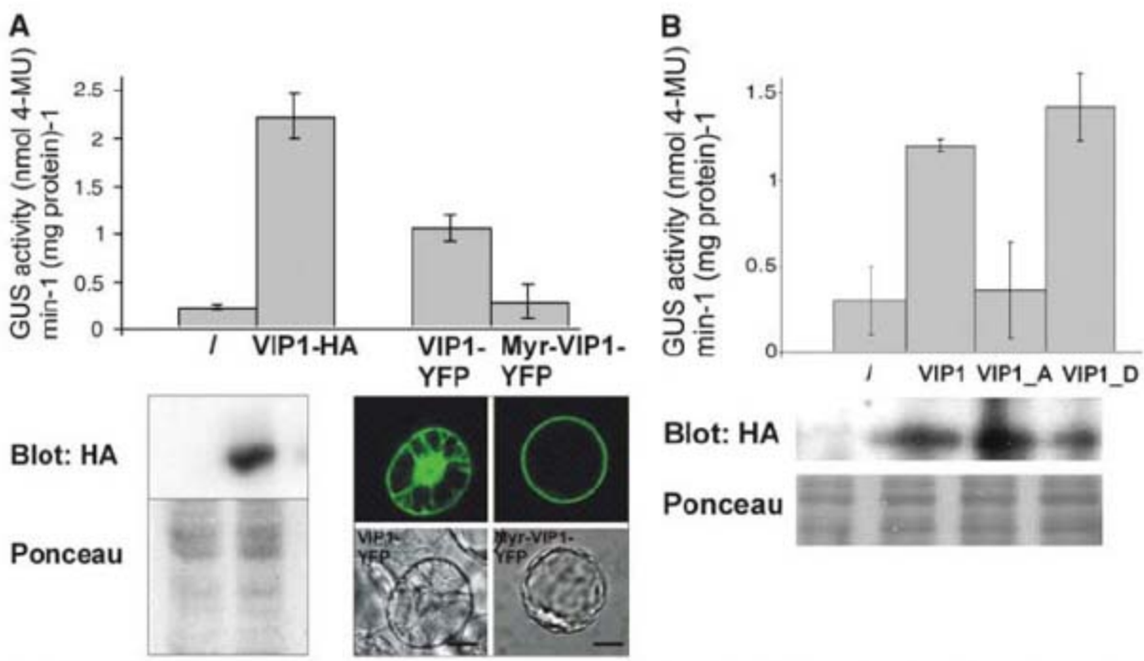


Fig. 3. (**A**) PR1 induction by nuclear VIP1. GUS activity in protoplasts 16 hours after transfection with a PR1 promoter::GUS construct alone or in combination with a VIP1-HA, VIP1-YFP, or Myr-VIP1-YFP construct is shown (n = 6 independent protoplast transfection assays; error bars indicate SD). Expression of HA and YFP fusion proteins is visualized by immunoblotting and confocal microscopy, respectively. Scale bar, 20 μM. (**B**) VIP1 phosphorylation is required for nuclear import of agrobacterial T-DNA. GUS activity in cultured *Arabidopsis* root cells after 3 days of incubation with the agrobacterial virE3-deficient reporter strain (35S::GUSintron) and/or an effector strain (35S::VIP1-Myc, VIP1_A-Myc, or VIP1_D-Myc) is shown. A representative of four independent experiment series is shown (n = 3 independent coincubation samples; error bars indicate SD). Lower panel: anti-Myc immunoblotted protein extracts.

cultures were incubated with an *Agrobacterium* reporter strain carrying a 35*S*::GUS construct and/or an effector strain carrying a 35*S*::VIP1-Myc, 35*S*::VIP1_A-Myc, or 35*S*::VIP1_D-Myc construct. Transformation efficiency was measured by quantitative GUS fluorescence after 3 days of incubation. Consistent with previous reports, *Agrobacterium* transformation efficiency increased greatly upon coexpression of VIP1 (Fig. 3B). However, this effect was observed only for wild-type VIP1 and VIP1_D, but not VIP1_A (Fig. 3B). The reason why constitutively nuclear-targeted VIP1_D can enhance T-DNA transfer is most likely because the continuous protein synthesis of VIP1 enables the T-DNA complex to shuttle directly with de novo–synthesized cytosolic VIP1_D to the nucleus. The possibility that the increased GUS activity in VIP1- and VIP1_D-expressing cells was due to a transcriptional activation of the 35*S* promoter can be excluded, because GUS activity in protoplasts transfected with a 35*S*::GUS reporter was unaffected by cotransfection with VIP1, VIP1_A, or VIP1_D (fig. S3). Overall, these results confirm previous reports (*6*, *14*) that the efficiency of *Agrobacterium* transformation can be enhanced by coexpression of VIP1, but show that this effect depends on the ability to phosphorylate and thereby shuttle VIP1 into the nuclear compartment.

Our work suggests a role for VIP1 as a transcription factor in host defense, whose subcellular localization is regulated by an *Agrobacterium*-induced MAPK cascade. We hypothesize that at some point in evolution, agrobacteria took advantage of being recognized by the host by encroaching on VIP1 as a T-DNA shuttle into the host nucleus (fig. S4). Future research should allow the identification of the complete set of target genes regulated by VIP1 in the plant and help to unravel the role of VIP1 in transcriptional regulation during pathogen defense.

References and Notes

1. T. Nurnberger, F. Brunner, B. Kemmerling, L. Piater, *Immunol. Rev.* **198**, 249 (2004).
2. A. Espinosa, J. R. Alfano, *Cell. Microbiol.* **6**, 1027 (2004).
3. Z. Nimchuk, T. Eulgem, B. F. Holt 3rd, J. L. Dangl, *Annu. Rev. Genet.* **37**, 579 (2003).
4. D. A. Jones, D. Takemoto, *Curr. Opin. Immunol.* **16**, 48 (2004).
5. A. C. Vergunst *et al.*, *Science* **290**, 979 (2000).
6. V. Citovsky *et al.*, *Cell. Microbiol.* **9**, 9 (2007).
7. H. Nakagami, A. Pitzschke, H. Hirt, *Trends Plant Sci.* **10**, 339 (2005).
8. T. Asai *et al.*, *Nature* **415**, 977 (2002).
9. T. Meszaros *et al.*, *Plant J.* **48**, 485 (2006).
10. T. S. Nuhse, S. C. Peck, H. Hirt, T. Boller, *J. Biol. Chem.* **275**, 7521 (2000).
11. T. Tzfira, M. Vaidya, V. Citovsky, *EMBO J.* **20**, 3596 (2001).
12. R. Y. Ma *et al.*, *J. Cell Sci.* **118**, 795 (2005).
13. J. Li, A. Krichevsky, M. Vaidya, T. Tzfira, V. Citovsky, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 5733 (2005).
14. T. Tzfira, M. Vaidya, V. Citovsky, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 10435 (2002).

15. We thank E. Scheikl, M. Teige, A. Auer, S. Schwarz, and A. Belokurov for assistance and P. Guggenthaler for the introduction to the confocal microscope. We thank P. Hooykaas (Leiden University, Netherlands) for providing us with the virE3-deficient and wild-type agrobacterial strains LBA1010, and C. Zipfel (John Innes Center, Norwich, UK) for providing the 35S::GUSintron_pBin construct. The work was supported by projects of the Austrian Science Fund FWF, the Vienna Science and Technology Fund, and the University of Vienna.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/453/DC1
Materials and Methods
Figs. S1 to S4
References

20 July 2007; accepted 29 August 2007
10.1126/science.1148110

Structure of a NHEJ Polymerase–Mediated DNA Synaptic Complex

Nigel C. Brissett,[1]* Robert S. Pitcher,[1]* Raquel Juarez,[2] Angel J. Picher,[2] Andrew J. Green,[1] Timothy R. Dafforn,[3] Gavin C. Fox,[4] Luis Blanco,[2]† Aidan J. Doherty[1]†

Nonhomologous end joining (NHEJ) is a critical DNA double-strand break (DSB) repair pathway required to maintain genome stability. Many prokaryotes possess a minimalist NHEJ apparatus required to repair DSBs during stationary phase, composed of two conserved core proteins, Ku and ligase D (LigD). The crystal structure of *Mycobacterium tuberculosis* polymerase domain of LigD mediating the synapsis of two noncomplementary DNA ends revealed a variety of interactions, including microhomology base pairing, mismatched and flipped-out bases, and 3′ termini forming hairpin-like ends. Biochemical and biophysical studies confirmed that polymerase-induced end synapsis also occurs in solution. We propose that this DNA synaptic structure reflects an intermediate bridging stage of the NHEJ process, before end processing and ligation, with both the polymerase and the DNA sequence playing pivotal roles in determining the sequential order of synapsis and remodeling before end joining.

DNA double-strand breaks (DSBs) are a potentially lethal form of cellular damage, and failure to repair such breaks can lead to genomic instability (*1*, *2*). In higher eukaryotes, the nonhomologous end joining (NHEJ) pathway is critical for the repair of DSBs (*1*). A functionally homologous repair system exists in many prokaryotes, where it is used to repair DSBs in stationary-phase and sporulating cells (*3*, *4*). The bacterial NHEJ complex is composed of two proteins, Ku and a multifunctional DNA ligase (LigD) (*3*–*10*). In addition to a core ligase domain, LigD often possesses ancillary polymerase (PolDom) and nuclease domains (*6*–*15*). PolDom, a member of the archaeo-eukaryotic primase (AEP) superfamily (*6*, *16*, *17*), in turn has a variety of nucleotidyl transferase activities (*6*–*9*) as well as the ability to generate template distortions and primer realignment (*12*, *13*). Here, we describe the crystal structure of a NHEJ polymerase–mediated synaptic complex, which reveals a DNA-directed mechanism used by repair polymerases to induce synapsis of noncomplementary ends through a dimeric arrangement.

PolDom can interact with a 3′-protruding DNA end containing a 5′-phosphate (5′-P) on the downstream strand (*12*), a probable first step in end joining, which is compatible with Ku binding near the ends. In the second step, PolDom may endeavor to connect the 3′-protruding DNA ends to configure a "gap-like" synaptic intermediate. When different DNA molecules were tested in polymerization assays, extension of the 3′-protruding strand by the *Mycobacterium tuberculosis* polymerase domain (*Mt*-PolDom) of LigD was observed by providing nucleoside triphosphates. However, template extension was restricted to the addition of a few nucleotides, suggesting that the specific nucleotides inserted may be templated, perhaps as a result of the pairing of the 3′ ends. In the DNA shown in Fig. 1A, the 3′-protruding nucleotide [deoxycytidine (dC)] is complementary to the base preceding the nucleotide (dC), which is adjacent to the 5′-P; therefore, a structure resembling a single-nucleotide gap can be formed, either by self-annealing (snap-back) or by connection of two ends (synapsis). In agreement with this mechanism, guanosine triphosphate (GTP) was preferentially incorporated by PolDom.

The specificity of the elongation reaction was analyzed using variants of the 3′-protruding oligonucleotide that differed in the deoxynucleotide (X) adjacent to the internal 5′-P. As predicted, the base preferentially added to the 3′-protruding strand varied as a function of the X nucleotide, which acts as a template (fig. S1). Having shown

[1]Genome Damage and Stability Centre, University of Sussex, Brighton BN1 9RQ, UK. [2]Centro de Biología Molecular Severo Ochoa, CSIC-UAM, 28049 Madrid, Spain. [3]Department of Biosciences, University of Birmingham, Birmingham B15 2TT, UK. [4]European Synchrotron Radiation Facility, LLS-BM16, Grenoble Cedex 9, France.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: lblanco@cbm.uam.es (L.B.); ajd21@sussex.ac.uk (A.J.D.)

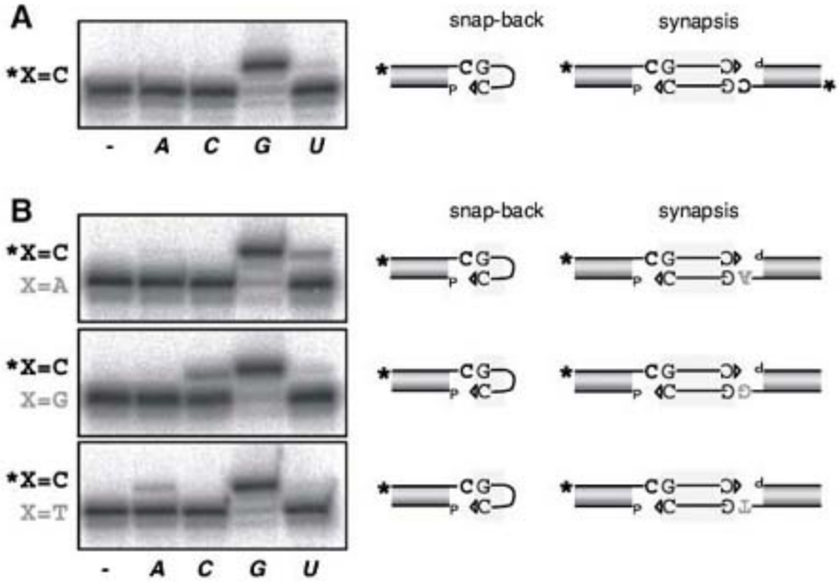


Fig. 1. PolDom promotes synapsis of two 3′-protruding ends. Ribonucleotide insertion at 3′-protruding ends was carried out in the presence of 400 nM *Mt*-PolDom and 100 μM of each individually added nucleotide triphosphate. After incubation for 30 min at 30°C, primer extension products were detected by autoradiography. (**A**) When using a labeled DNA molecule in which the neighboring nucleotide to the 5′-P (X) was dC, only the expected insertion (G) is observed; however, this does not distinguish between the alternative modes depicted ("snap-back" or "synapsis"). (**B**) By adding a second 3′-protruding end molecule (also having a 5′-P but unlabeled) that provides a different nucleotide neighbor to the 5′-P (X = A, G, or T), a second prominent insertion is observed in each case, complementary to the X base provided "in trans."

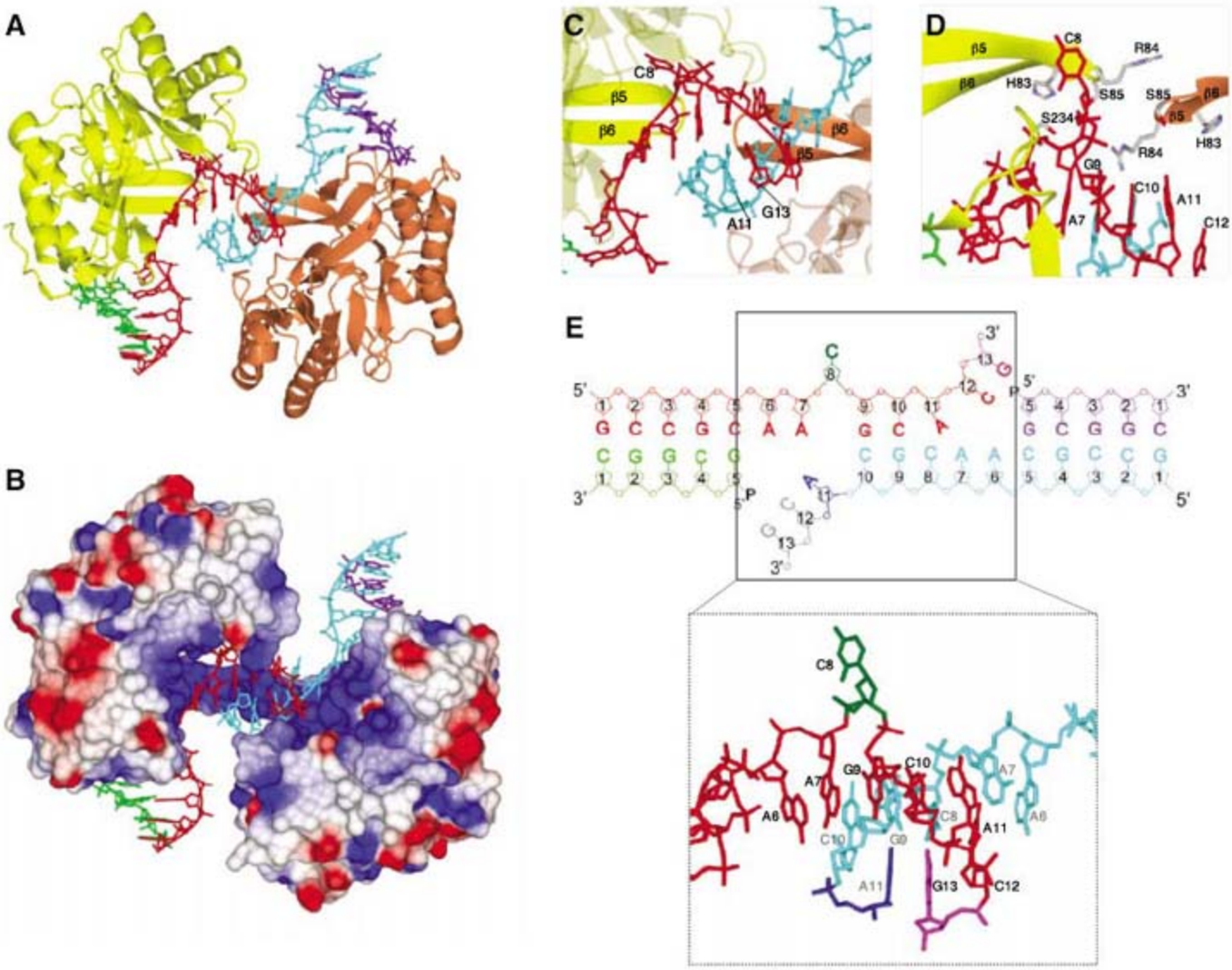


Fig. 2. Crystal structure of a polymerase-mediated DNA synaptic complex. (**A**) Ribbon representation of two *Mt*-PolDom–DNA binary complexes, in which each polypeptide and DNA chain is colored differently. (**B**) Electrostatic surface potential of the two PolDom monomers, emphasizing the symmetry of the synaptic complex and the positively charged atrium (blue area) nesting the synapsed 3′-ends. (**C**) Close-up view emphasizing the protruding β hairpin (loop 1) provided by each PolDom monomer (darker color) for dealing with the synapsis. (**D**) A view illustrating the residues (*22*) of loop 1 that bind to DNA in the synapsed region, in particular stabilizing the flipped-out base (C8) in the F chain (red). (**E**) Schematic view of the synapsed DNA, in which each DNA strand is differently colored as in (A) to (D). Nucleotides C12 and G13 of chain D (cyan) are depicted in light gray, as they are not seen in the crystal structure. The flipped-out base (C8 in chain F) is outlined in dark green. The bases A11 (chain D) and G13 (chain F), colored dark blue and magenta, respectively, form hairpin-like structures contributed by both 3′-ends.

that the templating nucleotide is adjacent to the 5′-P, we measured whether synapsis of two ends was actually occurring. By simultaneously adding two distinct DNA "ends" (only one labeled) that differed in the nucleotide adjacent to the 5′-P, we observed that extension of the labeled DNA end occurred with either of the two nucleotides complementary to each of the possible templating nucleotides (Fig. 1B). Incorporation of GTP could reflect the snap-back reaction, but incorporation of the other nucleotides into the labeled DNA end was only possible if synapsis occurred with the unlabeled DNA. This establishes that *Mt*-PolDom is capable of mediating the bridging of two DNA ends. This conclusion is also supported by analytical ultracentrifugation and protein cross-linking studies (fig. S2).

PolDom is a functionally independent domain of LigD (*11*), which also contains nuclease and ligase domains that can interact with the DNA. As with PolDom, LigD catalyzed the templated elongation of a 3′-protruding DNA end, which could subsequently be ligated (fig. S3A). A 5′-P also stimulated this templated reaction catalyzed by LigD (fig. S3A), confirming that phosphate recognition is also critical for LigD. Moreover, LigD catalyzed 3′-terminal additions that were templated by a distinct DNA end, providing biological relevance to the finding that synapsis is mediated by the polymerization domain of LigD (fig. S3B).

To understand the molecular basis for DNA end binding by a prokaryotic NHEJ polymerase/primase, we elucidated the crystal structure (2.4 Å) of *Mt*-PolDom in complex with DNA containing a 3′-overhang and a recessed 5′-P. The structure revealed two PolDom-DNA complexes connected via the 3′-protruding DNA ends, forming a NHEJ polymerase–mediated synaptic complex (Fig. 2A). PolDom contains a prominent surface β-hairpin structure, loop 1 (Fig. 2, A to C), which is specific to NHEJ AEPs such as *Mt*-LigD and *Pseudomonas aeruginosa* LigD (*Pa*-LigD) (fig. S4). Conserved residues on loop 1 interact with

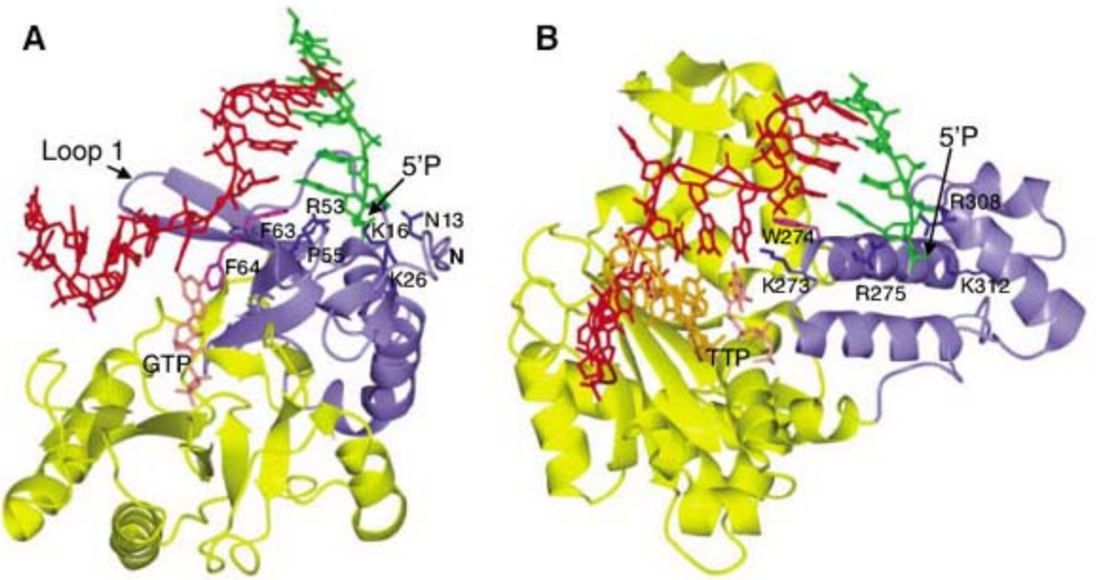


Fig. 3. Crystal structure of the *Mt*-PolDom monomer complexed with a 3′-protruding DNA end. (**A**) Ribbon representation of the *Mt*-PolDom complexed with a T/D molecule, emphasizing the most critical contacts or subdomains (loops) involved in DNA interaction. The first N-terminal 112 amino acids are colored in blue to outline domain similarities with the "8-kD" domain of Pol λ (*18*). The template strand (T) is shown in red and the downstream strand (D) in green. The location of an incoming GTP (pink; modeled from structure at PDB code 2IRX) supports the functional importance of the complex. (**B**) Ribbon representation of the ternary complex consisting of Pol λ, a single-nucleotide gap, and dideoxythymidine triphosphate (ddTTP) (PDB code 2BCV), emphasizing similar residues acting as DNA ligands at the downstream side of the gap; these are most likely critical for the NHEJ function of Pol λ. The region corresponding to the "8-kD" domain is depicted in blue. Red, template strand; orange, primer strand; green, downstream strand; pink, ddTTP.

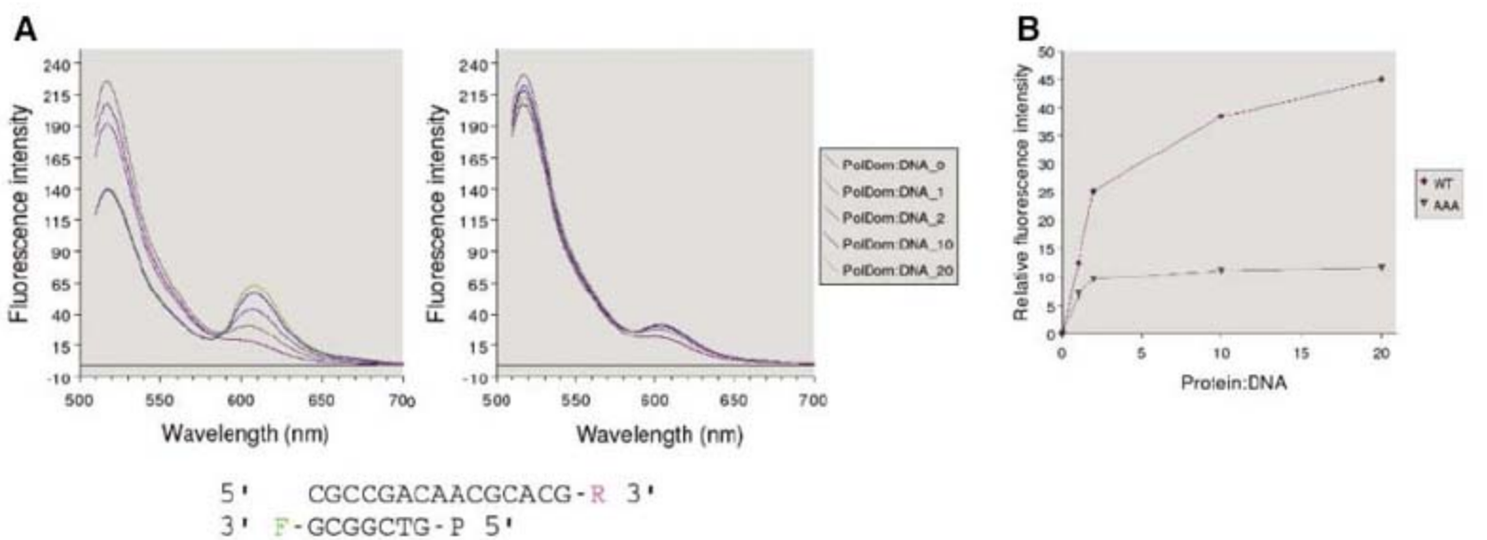


Fig. 4. PolDom-mediated DNA synapsis probed by FRET. (**A**) Steady-state fluorescence spectra of 3′-FAM (donor)/3′-ROX (acceptor)–labeled, 3′-protruding DNA with varying ratios of *Mt*-PolDom; ratios of PolDom to DNA are indicated at the right. The doubly labeled 3′-protruding DNA was incubated with increasing amounts of either wild-type (WT, left panel) or loop-mutant (*mut-loop*, right panel) *Mt*-PolDom. The presence of a FRET emission peak signifies a close approach of the 3′-overhang with the duplex portion of another DNA, mediated by PolDom. The difference in maximal FRET signal between WT and mutant protein indicates that the loop residues play a critical role in synaptic complex formation. (**B**) Relative FRET differences between WT and *mut-loop* (AAA) *Mt*-PolDoms in the presence of DNA (at ROX emission maxima, λ_{em} = 605 ± 2 nm) showing that, at a ratio of 20:1, WT PolDom exhibits a >4-fold increase in fluorescence emission due to FRET relative to the mutant PolDom-DNA. The DNA concentration is 50 nM.

the 3′-protrusion and orient the synapsis of the ends (Fig. 2D). The 3′-overhangs of the opposing template strands are brought together via a number of base-pairing and stacking interactions (Fig. 2E and fig. S6) (*18*). Each PolDom monomer makes intimate contact with the 5′-P on the downstream strand, which is bound in a positively charged pocket formed by Lys^{16} and Lys^{26} (Fig. 3A), two residues absolutely conserved in NHEJ AEPs (fig. S4). Notably, the N-terminal PolDom region containing Lys^{16} is absent in AEPs from Archaea and Eukarya (fig. S4).

The *Mt*-PolDom mutant (Lys^{16} → Ala) was unable to bind to DNA and had very reduced polymerase activity (fig. S5), whereas gap filling was normal. Other interactions with DNA are indicated in figs. S4 and S6. The PolDom-DNA interactions are reminiscent of the contacts observed in the structure of the evolutionary unrelated NHEJ polymerase, Pol λ–gapped DNA complex (*18*, *19*) (Fig. 3).

The apical loop 1 (β5-β6) interacts with the 3′-protruding strand, thus constituting a potentially important element for maintaining the synapsis between two 3′-protruding DNA ends (Fig. 2, C and D). To analyze the functional importance of these interactions, we mutated loop 1 residues (83 to 85) to alanine and evaluated the DNA binding and polymerization capacity of the resulting mutant (*mut-loop*). On a gapped DNA substrate, the DNA binding potential of *mut-loop* was equivalent to that of wild-type PolDom (fig. S7). Therefore, the presence of a 5′-P appears to be enough to ensure enzyme-DNA stability in a gap, and loop 1 is dispensable when the primer terminus, the template, and the 5′-P are physically connected and not discontinuous. However, the integrity of loop 1 was critical to forming a synaptic complex of two 3′-protruding DNAs. Electrophoretic mobility shift and 3′-extension assays showed that *mut-loop* was very inefficient at forming a synaptic complex (fig. S8). An analogous loop-like structure may play a related role in eukaryotic NHEJ polymerases (*20*, *21*).

The importance of PolDom, and loop 1 in particular, in mediating DNA synapsis was further probed by fluorescence resonance energy transfer (FRET) using DNA with a 3′-overhang identical to that present in the crystal structure. The steady-state fluorescence spectra of doubly labeled 3′-protruding DNA (3′-fluorescein donor and 3′-rhodamine acceptor) with increasing amounts of wild-type *Mt*-PolDom showed a marked concentration-dependent increase in emission of the rhodamine fluorophore at 605 nm (Fig. 4) due to FRET from fluorescein. The presence of a PolDom-dependent FRET emission peak signifies a close approach of the 3′-overhang with the duplex region of another DNA, indicative of a stable protein-mediated interaction between two DNA ends. In contrast, the *mut-loop* mutant exhibited a markedly reduced FRET signal, indicating that loop 1 plays a critical role in stabilizing the synaptic complex. This conclusion is further supported by protein cross-linking studies (fig. S2).

The structure presented here establishes that NHEJ polymerases can promote the formation of end-bridging complexes, thereby directing the break alignment process (fig. S9). The limited number of contacts made between the enzyme and the 3′-protrusions suggests that PolDom, and presumably other NHEJ polymerases, allow a large degree of rotational freedom that enables the termini to search for sequence complementarities on the opposing break. This "homology" searching process acts, together with Ku, to align the break by forming presynaptic bridging structures, promoted by favorable microhomology-directed base pairing, that nucleate the formation of the synaptic complex (fig. S9). Thus, final end synapsis, like that shown in the crystal structure, may require a certain degree of mispairing, template dislocation, or realignment facilitated by base flipping, and the eventual formation of hairpin structures at the terminal ends. The hairpin-like structures observed—located in a large, solvent-accessible channel within the PolDom complex—could conceivably accommodate the small 3′-exonuclease domain of LigD (NucDom) (*6*, *15*), facilitating the controlled resectioning of the ends. This may possibly explain the preference of NucDom for recessed 3′-ends (*6*, *15*) and suggests that the nuclease resection process may be regulated by the conformation of the ends within the synaptic complex.

References and Notes

1. L. Krejci, L. Chen, S. Van Komen, P. Sung, A. Tomkinson, *Prog. Nucleic Acid Res. Mol. Biol.* **74**, 159 (2003).
2. J. M. Daley, P. L. Palmbos, D. Wu, T. E. Wilson, *Annu. Rev. Genet.* **39**, 431 (2005).
3. G. R. Weller *et al.*, *Science* **297**, 1686 (2002).
4. R. S. Pitcher *et al.*, *DNA Repair* **6**, 1271 (2007).
5. R. S. Pitcher *et al.*, *Mol. Cell* **23**, 743 (2006).
6. M. Della *et al.*, *Science* **306**, 683 (2004).
7. R. S. Pitcher, T. E. Wilson, A. J. Doherty, *Cell Cycle* **4**, 675 (2005).
8. R. Bowater, A. J. Doherty, *PLOS Genet.* **2**, 93 (2006).
9. C. Gong, A. Martins, P. Bongiorno, M. Glickman, S. Shuman, *J. Biol. Chem.* **279**, 20594 (2004).
10. C. Gong *et al.*, *Nat. Struct. Mol. Biol.* **12**, 304 (2005).
11. R. S. Pitcher, L. M. Tonkin, A. J. Green, A. J. Doherty, *J. Mol. Biol.* **351**, 531 (2005).
12. R. S. Pitcher *et al.*, *J. Mol. Biol.* **366**, 391 (2007).
13. L. Yakovleva, S. Shuman, *J. Biol. Chem.* **281**, 25026 (2006).
14. H. Zhu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 1711 (2006).
15. H. Zhu, S. Shuman, *J. Biol. Chem.* **281**, 13873 (2006).
16. L. M. Iyer, E. V. Koonin, D. D. Leipe, L. Aravind, *Nucleic Acids Res.* **33**, 3875 (2005).
17. S. H. Lao-Sirieix, L. Pellegrini, S. D. Bell, *Trends Genet.* **21**, 568 (2005).
18. See supporting material on *Science* Online.
19. M. Garcia-Diaz *et al.*, *Cell* **124**, 331 (2006).
20. S. A. Nick McElhinny *et al.*, *Mol. Cell* **19**, 357 (2005).
21. R. Juárez, J. F. Ruiz, S. Nick McElhinny, D. A. Ramsden, L. Blanco, *Nucleic Acids Res.* **34**, 4572 (2006).

22. Abbreviations for amino acid residues: F, Phe; H, His; K, Lys; N, Asn; P, Pro; R, Arg; S, Ser; W, Trp.

23. We thank T. Brown, A. Lebedev, M. Osbourne, and D. Thompson for technical advice and support; T. Wilson for valuable comments on the manuscript; and the European Synchrotron Radiation Facility for provision of synchrotron radiation facilities. Work in A.J.D.'s laboratory is supported by grants from the UK Biotechnology and Biological Sciences Research Council, Cancer Research UK, the Association for International Cancer Research, and the Royal Society. Work in L.B.'s laboratory was supported by Ministerio de Ciencia y Tecnología grant BMC 2003-00186, Consolider CSD2007-00015, and an institutional grant to Centro de Biología Molecular Severo Ochoa from Fundación Ramón Areces. A.J.P. is a recipient of a fellowship from the Spanish Ministry of Science and Technology.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/456/DC1

Materials and Methods
SOM Text
Figs. S1 to S9
Table S1
References

15 May 2007; accepted 14 September 2007
10.1126/science.1145112

Structure of Hexameric DnaB Helicase and Its Complex with a Domain of DnaG Primase

Scott Bailey,[1] William K. Eliason,[1] Thomas A. Steitz[1,2]*

The complex between the DnaB helicase and the DnaG primase unwinds duplex DNA at the eubacterial replication fork and synthesizes the Okazaki RNA primers. The crystal structures of hexameric DnaB and its complex with the helicase binding domain (HBD) of DnaG reveal that within the hexamer the two domains of DnaB pack with strikingly different symmetries to form a distinct two-layered ring structure. Each of three bound HBDs stabilizes the DnaB hexamer in a conformation that may increase its processivity. Three positive, conserved electrostatic patches on the N-terminal domain of DnaB may also serve as a binding site for DNA and thereby guide the DNA to a DnaG active site.

Most DNA polymerases, unlike RNA polymerases, are unable to unwind duplex DNA and require a primed single-stranded DNA (ssDNA) substrate to initiate DNA synthesis. In eubacterial cells, these functions are performed by a complex of the DnaB helicase and the DnaG primase (*1*). DnaB unwinds the duplex DNA fueled by the hydrolysis of nucleoside triphosphate (NTP) (*2*), whereas DnaG uses the newly formed

ssDNA as a template for the de novo synthesis of RNA primers (*1*). DnaB oligomerizes into a homo-hexameric ring that has been observed by electron microscopy (EM) to form either six-fold or three-fold symmetry states (*3*, *4*). The DnaB ring is thought to unwind DNA at the replication fork by translocating along and encircling the 5′ lagging strand, while the 3′ leading strand is occluded (*5*, *6*). The crystal structure of a monomer of DnaB has revealed that the helicase is composed of two domains separated by a flexible linker (*7*). The C-terminal domain (CTD) forms a RecA-like fold that contains the NTP and DNA binding sites, whereas the N-terminal domain (NTD) is composed of a helical bundle terminated by an extended helical hairpin (*7*). Although the NTD is required for helicase activity (*8*–*10*) and may define the direction of movement of the helicase on DNA (*10*), its precise function in DNA unwinding is not clear.

The interaction between DnaB and DnaG stimulates both of their activities. DnaG increases both the NTPase and the helicase activities of DnaB (*9*), and DnaB both increases and modulates the synthesis of RNA primers by DnaG (*1*). DnaG consists of three domains, an N-terminal zinc-binding domain (ZBD), an RNA polymerase domain (RPD), and a C-terminal HBD. The HBD of DnaG, whose ternary structure consists of a helical bundle (the C1 subdomain) terminated by a helical hairpin (the C2 subdomain), is sufficient to both bind to and stimulate the activities of DnaB (*9*, *11*). The tertiary structure of the HBD is highly similar to the fold of the NTD of DnaB (*7*, *12*, *13*). The stability of the interaction between DnaB and DnaG varies substantially among species. In *Escherichia Coli*, the interaction is relatively weak and can only be detected by sensitive techniques (*13*–*15*), whereas DnaB and DnaG from *Bacillus stearothermophilus* (*Bst*) form a tight interaction that persists when the complex is run over a gel filtration column (*9*). Despite these differences in the stability of their complexes, the biochemical behavior of DnaB and DnaG from *E. coli* and *Bst* are similar (*16*).

We have obtained two crystal structures of unliganded hexameric *Bst* DnaB (crystal forms B1 and B2) and two crystal structures of DnaB in complex with the HBD of DnaG (forms BH1 and BH2). These four crystal forms diffract x-rays to between 5.0 and 2.9 Å resolution (Table 1). Experimental phases were determined separately for each crystal form, either by the single-wavelength anomalous diffraction method using selenomethionine-substituted protein or by heavy-atom derivative methods using crystals that had been soaked in solutions containing mercury chloride (*17*). Phasing of the diffraction from each crystal form and the resulting electron density maps (Fig. 1A) were substantially improved by cross-crystal symmetry averaging among all four crystal forms (*17*). Data collection and refinement statistics for each crystal form are in Table 1. The structures of DnaB presented here differ only in the relative orientation of their CTDs. Therefore, unless otherwise stated, discussion will focus on the 2.9 Å resolution structure of DnaB complexed with HBD (form BH1), which has been refined to a free R-factor of 29.8%.

The two domains of the DnaB hexamers form a distinct double-layered ring structure in which the NTDs (residues 1 to 152) pack into a rigid triangular collar seated on top of a more loosely packed ring of CTDs (residues 186 to 454) (Fig. 1, B and C). Adjacent NTDs adopt one of two conformations that place their helical hairpins (residues 102 to 151) either on top of a neighboring CTD or on top of their own CTD (Fig. 1, C and D). These two conformations result in the six NTDs forming a trimer of head-to-head dimers related by two-fold symmetry in which three of the alternately oriented NTDs face the central channel of the ring (Fig. 1C). This trimer-of-dimers is stabilized by the hydrophobic interface that buries 2300 Å^2

Table 1. Data Collection and Refinement Statistics

Crystal Form	B1	B2	BH1	BH2
Spacegroup	$P2_12_12$	R32	P321	$P3_121$
Unit cell a,b,c (Å)	371, 110, 113	200, 200, 195	229, 229, 193	230, 230, 193
Resolution (Å)	50 – 3.7	50 – 5.0	50 – 2.9	50 – 4.0
Rmerge (%)*	8.1 (59.0)	5.8 (53.3)	7.0 (>100)	10.3 (50.3)
Completeness (%)*	97.3 (86.6)	95.8 (74.5)	99.8 (99.9)	99.9 (100.0)
I/σI*	15.0 (1.4)	35.9 (3.5)	22.3 (1.9)	19.6 (3.6)
Rwork*	30.8 (36.0)	39.4 (48.3)	25.9 (32.4)	32.0 (34.0)
Rfree*	32.3 (35.8)	39.7 (49.1)	29.7 (40.0)	34.4 (38.4)
RMSD† bond (Å)/angle (°)	0.009/1.416	–/–	0.009/1.389	0.010/1.659

*Values in parentheses correspond to the last resolution shell. †Root mean square deviation.

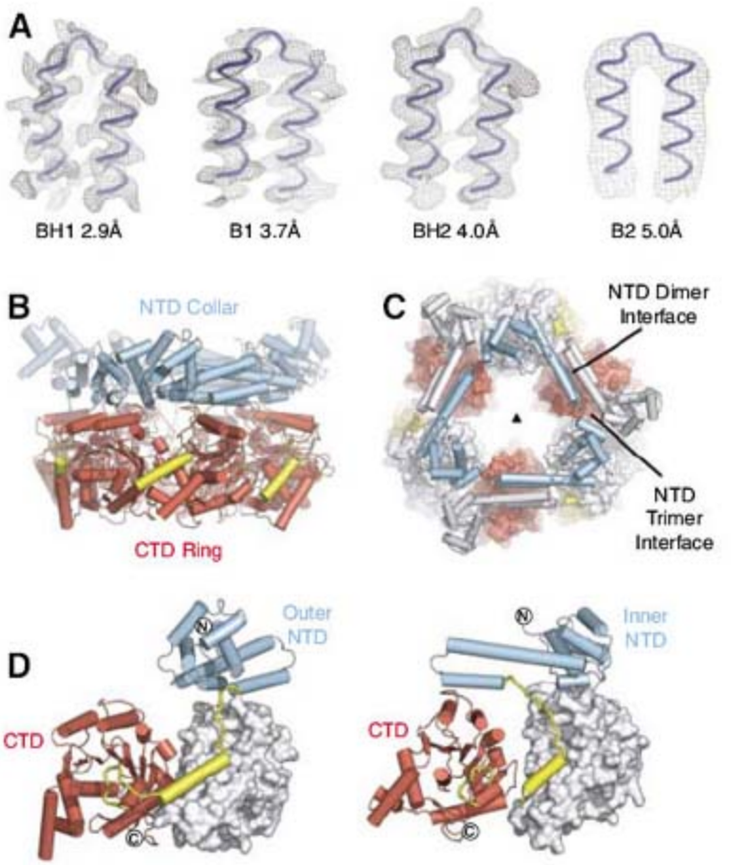


Fig. 1. Architecture of the DnaB hexamer. **(A)** Experimentally phased and cross-crystal averaged electron density maps of the four DnaB crystal forms. Shown at the foot of each map is the high-resolution limit at which each map was calculated. **(B)** "Side" view, orthogonal to the ring axis, of a ribbon representation of the DnaB hexamer. The NTD, CTD, and linker region are colored blue, red, and yellow respectively. **(C)** "Top" view, looking down the ring axis, of the DnaB hexamer. The CTDs are shown in a surface representation; the NTDs are shown as ribbons. Those subunits whose NTDs lie on the inner surface of the ring are colored as in (B), and those on the outer surface of the ring are colored white. **(D)** "Side" view of the two distinct conformations of the DnaB subunits within the hexamer, colored as in (B). Adjacent CTDs interacting with the linker region are shown as white surface representations.

[1]Department of Molecular Biophysics and Biochemistry and Howard Hughes Medical Institute, Yale University, New Haven, CT 06520, USA. [2]Department of Chemistry, Yale University, New Haven, CT 06520, USA.

*To whom correspondence should be addressed. E-mail: eatherton@csb.yale.edu

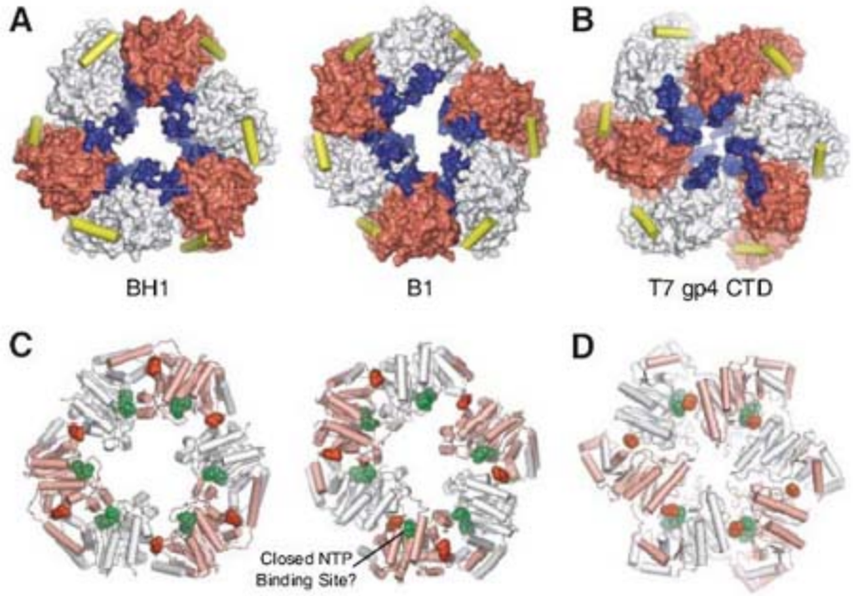


Fig. 2. Structure of the CTD ring. **(A)** Surface representation of the CTD rings of crystal forms BH1 (left) and B1 (right). Alternate subunits are colored white and red. The predicted DNA binding loops are colored blue, and the linker helices are shown as yellow cylinders. **(B)** The structure of the T7 gp4 helicase domain (*23*), displayed as in panel (A). **(C)** Ribbon representations of the CTD rings of crystal forms BH1 (left) and B1 (right). Alternate subunits are colored white and pink. NTP modeled at the six potential NTP binding sites of DnaB (22) are shown as green spheres; the Arginine fingers (Arg^{420}) are displayed as red spheres. **(D)** The structure of the T7 gp4 hexamer with four NTD binding sites occupied, displayed as in (C).

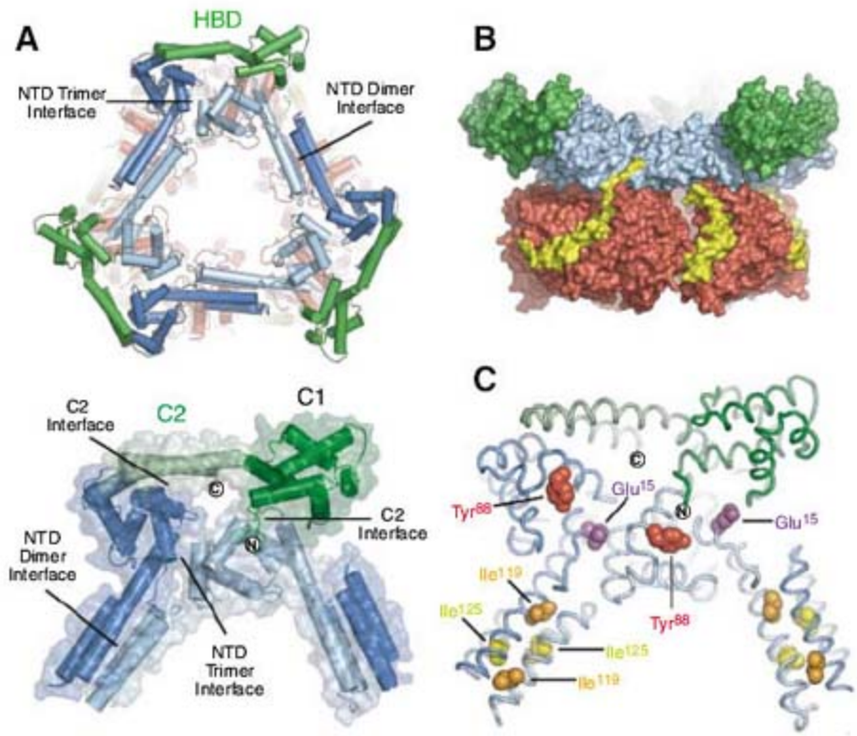


Fig. 3. Structure of the complex between DnaB and HBD. **(A)** (Top) "Top" view of a ribbon representation of the complex showing the three HBDs (green) bound at the periphery of the NTD collar (light blue and blue). The CTD and linker region are colored red and yellow, respectively. (Bottom) The interface between DnaB and HBD shown as ribbons with a transparent surface. **(B)** "Side" view of a surface representation of the complex revealing no interaction between the HBDs (green) and the DnaB CTD (red) or linker region (yellow). **(C)** Backbone trace of the HBD DnaB interface, residues known to modulate the interaction between DnaB and DnaG, are shown as colored spheres.

within the dimers and by the hydrophobic interface between dimers that buries 1050 $Å^2$ total surface area. Formation of the NTD collar appears to be highly cooperative, requiring the presence of the CTD, because truncated protein containing the NTD alone forms either monomers or dimers (*9*). Despite differences in crystal packing interactions of the NTDs, the structures of the NTD collar are similar in all four crystal forms (fig. S1).

Comparison of the structures of the four DnaB hexamers shows that their CTDs adopt a variety of different orientations around the ring but still bind the linker helix (residues 162 to 178) of the adjacent subunit at the periphery of the hexamer and orient their proposed DNA binding loops (*18*) toward the central channel (Fig. 2A and fig. S2). With the exception of the B1 structure, in which the CTDs form a distinctly irregular ring with no rotational symmetry, all of the CTD rings exhibit either exact or approximate three-fold symmetry (Fig. 2A and fig. S2). The hexameric rings of CTDs are held together primarily by their interactions within the linker region and not by interactions between adjacent CTDs. The interaction surface between adjacent CTDs in the four crystal structures ranges from little or none to 1100 $Å^2$ of total surface area, whereas the interface between each subunit and the linker region bury 2250 $Å^2$ of surface area (Fig. 1D). In addition to these interactions, the CTD ring appears to be additionally stabilized by the interactions between the NTDs, because mutants lacking the NTD have reduced hexamer stability (9, *19*).

The DnaB hexamer assembly has an outer diameter of 115 Å and a height of 75 Å. The diameter of the central channel through the NTD collar is ~50 Å, whereas the different orientations of the CTD rings result in channel diameters that vary between ~25 Å and ~50 Å in the four crystal structures. Thus, in the absence of its substrates, the diameter of the central channel of DnaB is wide enough to accommodate duplex DNA. Currently the crystal structure of the papillomavirus E1 helicase complexed with ssDNA and ADP (*20*) is the only high-resolution structure of a hexameric helicase bound to DNA. Because the E1 helicase uses different regions of its RecA-like domains for hexamer formation and DNA binding (*7*), the structure of DnaB bound to ssDNA must differ. However, it has been suggested that the spiral conformation of the bound ssDNA observed in E1 helicase structure may be common to all hexameric helicases (*20*). Fluorescence titration experiments have shown that DnaB binds ssDNA with a site size of ~20 bases (*21*). If the average distance between bases is 3.5 Å in the complex with DnaB, as was observed for E1 helicase (*20*), the DNA would extend ~70 Å, consistent with the 75 Å height of hexameric DnaB structures. The shape and dimensions of the crystal structures of DnaB are inconsistent with the shape and dimensions of the three-dimensional (3D) EM reconstructions of *E. coli*

DnaB (*3*) and G40P (*4*) (fig. S3); however, they are consistent with the 2D EM projections used to generate the 3D reconstructions. Therefore, it seems likely that the differences between the crystal and 3D EM data could be due to distortions generated by the negative staining process or to the methodological difficulties of generating 3D reconstructions of molecules as flexible as DnaB (discussed further in supporting online text).

The T7 gp4 protein of phage T7 contains two domains, one responsible for the helicase activity and the other for the primase activity, which are necessary for the replication of the T7 phage (*22*). Although the CTD of gp4 is homologous to the CTD of DnaB, gp4 lacks a domain equivalent to the DnaB NTD (*7*, *23*). Instead, two domains related to the ZPD and RPD of DnaG are fused to the N terminus of the protein. The crystal structure of the hexameric CTD of gp4 bound to a nonhydrolysable NTP analog (*18*) shows that the NTP binding pockets are formed between adjacent CTDs (Fig. 2, B and D). The majority of each pocket is formed by one CTD, whereas the adjacent CTD provides an arginine residue, the arginine finger, whose guanadinium group contacts the γ-phosphate of the bound NTP. The arginine finger is believed to stimulate NTP hydrolysis and to help modulate the relative orientation of the CTDs in response to NTP hydrolysis (*18*, *24*).

Comparison of the structures of DnaB and the gp4 helicase shows that the oligomerization of the two proteins is facilitated by a similar linker helix (Fig. 2), but the contacts between the adjacent CTDs of gp4 are much more extensive than those seen in DnaB. These more extensive contacts are generated by a rotation of the gp4 CTDs, both toward and about the plane of the hexamer ring, and result in a tighter gp4 hexamer that contains a central channel only wide enough to accommodate ssDNA (Fig. 2). The larger diameter of the central channels observed in the DnaB structures is probably due to the absence of bound NTP (supporting online text), because a more extended conformation of DnaB has been observed by EM in the absence of nucleotide (*3*). In addition, as a result of the orientations of the DnaB CTDs, most of the NTP binding sites are not near an arginine finger (Fig. 2C). Only the B1 structure has one pair of adjacent CTDs that position an arginine finger close enough to interact with the γ-phosphate of a bound NTP (Fig. 2C). This interface maybe representative of a nucleotide-bound state of DnaB. However, homology between the CTDs of DnaB and gp4 and the fact that superimposing the structure of the DnaB CTD onto the six CTDs of the nucleotide-bound gp4 structure produces a model with no steric clashes (*7*) suggest that the complex with nucleotide will orient the CTDs of DnaB to form a central channel that is only wide enough to accommodate ssDNA. The ability of DnaB to modulate the diameter of its central channel is, however, consistent with the observation that DnaB can translocate on ssDNA even when a complementary strand is present within the central channel (*25*).

Three molecules of the HBD are bound to the NTD collar of the DnaB hexamer in the structures of the complex (Fig. 3A). This stoichiometry agrees with analytical ultracentrifugation and gel filtration studies of the complex between *Bst* DnaB and DnaG, which show that between two and three molecules of DnaG bind to each DnaB hexamer (*9*). This number is also consistent with fluorescence anisotropy and cross-linking experiments conducted using the complex of *E. coli* DnaB and DnaG (*15*). The bound HBDs do not interact with either the CTD or the linker region of DnaB (Fig. 3B); consequently, there is no correlation between HBD binding and the position of the CTDs or the diameter of the CTD rings in the four structures presented here. The C2 subdomains of the HBD pack against the NTDs that form the outer surface of the ring while the C1 subdomains interact with the NTDs that form the inner surface of the ring (Fig. 3). Both interfaces are formed by a mixture of hydrophobic and polar contacts and each burry ~1200 Å^2 of total surface area (fig. S4). The C-terminal helix of the HBD (residues 577 to 595 in *Bst*) forms the majority of the interface with the C2 subdomain which is consistent with mutagenesis studies of *E. coli* DnaB (*11*). Overall, the interface with the C1 subdomain is less tightly packed than that with the C2 subdomain, perhaps explaining why the isolated C2 subdomains can form a gel filterable complex with DnaB, whereas the isolated C1 subdomain cannot (*12*). The C2 subdomain of *E. coli* DnaB is smaller than that of Bst DnaB, which also may explain why Bst DnaB and DnaG form a more stable complex than their *E. coli* counterparts (for more details, see supporting online text). The binding of one molecule of HBD to two NTD dimers effectively fixes the three-fold arrangement of the NTD collar (Fig. 3A), consistent with previous atomic force microscopy results (*26*).

Mutation of residues Tyr^{88}, Ile^{119}, or Ile^{125} in *Bst* DnaB (*16*, *26*) and of the equivalent residues in *E. coli* (*27*–*29*) and *Salmonella typhimurium* (*30*) inhibits the formation of a complex between DnaB and DnaG. Because Tyr^{88} lies near the interface with the HBD but does not directly contact it (Fig. 3C), this mutation presumably disrupts the tertiary structure of the NTD helical bundle. Residues Ile^{119} and Ile^{125} are buried from solvent at the NTD dimer interface (Fig. 3C), which suggests that their mutation would disrupt dimerization of the NTD. Hence, inhibition of DnaG binding by these mutants would appear to result from the destabilization of the trimer-of-dimers arrangement of the DnaB NTDs. Indeed, it has already been suggested that these mutant helicases may have altered NTD positions (*27*). Mutation of Glu^{15} in *Bst* DnaB has no effect on its binding to DnaG but does modulate the length of the primers synthesized by DnaG (16). The equivalent mutation in *E. coli* and *S. typhimurium* inhibits the binding of DnaG (*27*, *30*). Glu^{15} lies both at the C1 subdomain binding site and at the NTD trimer interface (Fig. 3C), consistent with its having a role in the formation of a DnaG complex. How this residue modulates primer synthesis in the complex of *Bst* DnaB and DnaG is currently not clear.

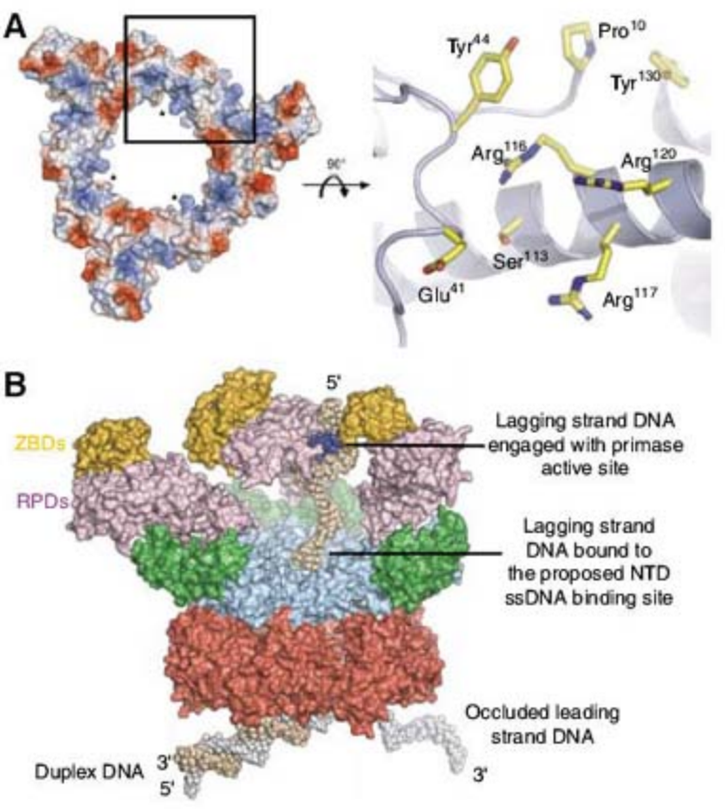


Fig. 4. DNA interactions. **(A)** (Left) "Top" view of a surface representation of the NTD collar colored blue for positive and red for negative electrostatic potentials. An asterisk highlights the proposed ssDNA binding sites. (Right) A detailed "side" view of the proposed ssDNA binding site boxed in (A). **(B)** Speculative model of DnaB complexed with DnaG and replication fork DNA. The proteins are shown in a surface representation (DnaB NTD, light blue; DnaB CTD, red; DnaG HBD, green; DnaG RPD, pink; and DnaG ZBD, orange). The modeled DNA is shown as white- and wheat-colored spheres; the RNA primer is shown in dark blue.

The binding of DnaG to DnaB stimulates the activities of DnaB (*1*) and stabilizes the three-fold conformation of the DnaB NTDs. This suggests that the three-fold symmetric state represents an activated form of DnaB; therefore, it seems doubtful that the DNA translocation mechanism of DnaB involves transitions between six- and three-fold symmetries. Both DnaB and the T7 gp4 proteins require a stable hexamer for NTPase and helicase activity (*9*, *18*). Therefore, the DnaG-mediated stimulation of the activities of DnaB could also result from the increased stability of the hexamer produced by the binding of DnaG, which is consistent with the observation that although the isolated C2 subdomain of the HBD can bind DnaB, both subdomains of the HBD are required for the stimulation of the activities of DnaB (16). Although the presence of DnaG at the replication fork in *E. coli* has been shown to be distributive (*31*), the binding of only one molecule of DnaG to DnaB would be sufficient to stabilize the three-fold conformation of DnaB. The closed circular structure of the NTD collar could also contribute to the stimulation of the helicase activity by keeping the two ssDNA strands topologically separated during unwinding. In addition the topological linking of DnaB to the DNA also would ensure that the two molecules could not easily disengage, thus increasing the processivity of the reaction. Kinetic analysis has shown that isolated DnaB is only a moderately processive enzyme, and it is assumed that it gains the processivity needed to replicate the genome from other components of the replication fork (*32*). A similar processivity role has also been suggested for the unrelated NTD of the papillomavirus E1 helicase (*20*).

The NTD collar may also provide an additional binding site for ssDNA. The interior surface of the NTD collar exhibits three distinct sites of positive electrostatic potential separated by regions of negative electrostatic potential (Fig. 4A). These positive sites are consistent with their binding DNA, contain residues that are conserved across DnaB species, and are well positioned for binding the ssDNA as it emanates from the CTD ring (Fig. 4). Nuclease protection and fluorescence energy transfer studies have also suggested the presence of a second ssDNA binding site at the N terminus of DnaB (*33*).

It is now possible to construct a model of the complex between DnaB and DnaG that illuminates how they cooperatively work together and stimulate each other's activities. The N terminus of each HBD is situated adjacent to the central channel of DnaB (Fig. 3), thereby positioning the N-terminal ZBD and RPD of full-length DnaG directly above the central channel (Fig. 4B). Thus, the structure of the RPD-ZBD fragment (*34*) can be positioned relative to the HBD in a manner that orients the primase active site with the proposed N-terminal ssDNA binding site of DnaB and is consistent with the structure of the truncated T7 gp4 helicase-primase heptamer (*21*). The structure of the complex between DnaB and HBD, and our modeling of the complex between the full-length proteins, is consistent with the possibility that DnaB stimulates the activity of DnaG by increasing the local concentration of the ssDNA substrate and by ensuring that multiple DnaG subunits are in close proximity to each other (*35*) (Fig. 4B). The latter is important because the RPD and ZBD function have been shown to function in trans, with each domain provided by a separate subunit (*35*).

References and Notes

1. J. E. Corn, J. M. Berger, *Nucleic Acids Res.* **34**, 4082 (2006).
2. J. H. LeBowitz, R. McMacken, *J. Biol. Chem.* **261**, 4738 (1986).
3. S. Yang *et al.*, *J. Mol. Biol.* **321**, 839 (2002).
4. R. Nunez-Ramirez *et al.*, *J. Mol. Biol.* **357**, 1063 (2006).
5. M. J. Jezewska, S. Rajendran, D. Bujalowski, W. Bujalowski, *J. Biol. Chem.* **273**, 10515 (1998).
6. D. L. Kaplan, *J. Mol. Biol.* **301**, 285 (2000).
7. S. Bailey, W. K. Eliason, T. A. Steitz, *Nucleic Acids Res.* **35**, 4728 (2007).
8. N. Nakayama, N. Arai, Y. Kaziro, K. Arai, *J. Biol. Chem.* **259**, 88 (1984).
9. L. E. Bird, H. Pan, P. Soultanas, D. B. Wigley, *Biochemistry* **39**, 171 (2000).
10. P. Mesa, J. C. Alonso, S. Ayora, *J. Mol. Biol.* **357**, 1077 (2006).
11. K. Tougu, K. J. Marians, *J. Biol. Chem.* **271**, 21391 (1996).
12. K. Syson, J. Thirlway, A. M. Hounslow, P. Soultanas, J. P. Waltho, *Structure* **13**, 609 (2005).
13. A. J. Oakley *et al.*, *J. Biol. Chem.* **280**, 11495 (2005).
14. Y. B. Lu, S. Bhattacharyya, M. A. Griep, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12902 (1996).
15. A. V. Mitkova, S. M. Khopde, S. B. Biswas, *J. Biol. Chem.* **278**, 52253 (2003).
16. J. Thirlway, P. Soultanas, *J. Bacteriol.* **188**, 1534 (2006).
17. Materials and methods are available as supporting material on *Science* Online.
18. M. R. Singleton, M. R. Sawaya, T. Ellenberger, D. B. Wigley, *Cell* **101**, 589 (2000).
19. S. B. Biswas, P. H. Chen, E. E. Biswas, *Biochemistry* **33**, 11307 (1994).
20. E. J. Enemark, L. Joshua-Tor, *Nature* **422**, 270 (2006).
21. W. Bujalowski, M. J. Jezewska, *Biochemistry* **34**, 8513 (1995).
22. E. A. Toth, Y. Li, M. R. Sawaya, Y. Cheng, T. Ellenberger, *Mol. Cell* **12**, 1113 (2003).
23. M. R. Sawaya, S. Guo, S. Tabor, C. C. Richardson, T. Ellenberger, *Cell* **99**, 167 (1999).
24. D. J. Crampton, S. Guo, D. E. Johnson, C. C. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 4373 (2004).
25. D. L. Kaplan, *J. Mol. Biol.* **301**, 285 (2000).
26. J. Thirlway *et al.*, *Nucleic Acids Res.* **32**, 2977 (2004).
27. L. Stordal, R. Maurer, *J. Bacteriol.* **178**, 4620 (1996).
28. P. Chang, K. J. Marians, *J. Biol. Chem.* **275**, 26187 (2000).
29. Y. B. Lu, P. V. A. L. Ratnakar, B. K. Mohanty, D. Bastia, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 12902 (1996).
30. R. Maurer, A. Wong, *J. Bacteriol.* **170**, 3682 (1988).
31. C. A. Wu, E. L. Zechner, K. J. Marians, *J. Biol. Chem.* **267**, 4030 (1992).
32. R. Galletto, M. J. Jezewska, W. Bujalowski, *J. Mol. Biol.* **343**, 83 (2004).
33. M. J. Jezewska, S. Rajendran, W. Bujalowski, *J. Biol. Chem.* **273**, 9058 (1998).
34. J. E. Corn, P. J. Pease, G. L. Hura, J. M. Berger, *Mol. Cell* **20**, 391 (2005).
35. S. J. Lee, C. C. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 12703 (2002).

36. This research was supported by NIH grant GM57510 to T.A.S. We thank P. Soultanas for the expression plasmids. Coordinates and structure factors of crystal forms BH1, BH2, B1, and B2 have been deposited under accession codes 2R6A, 2R6C, 2R6D, and 2R6E, respectively.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/459/DC1
Materials and Methods
SOM Text
Figs. S1 to S7
Tables S1 and S2
References

3 July 2007; accepted 11 September 2007
10.1126/science.1147353

Network Analysis of Oncogenic Ras Activation in Cancer

Edward C. Stites,[1,2,3] Paul C. Trampont,[1] Zhong Ma,[1] Kodi S. Ravichandran[1]*

To investigate the unregulated Ras activation associated with cancer, we developed and validated a mathematical model of Ras signaling. The model-based predictions and associated experiments help explain why only one of two classes of activating Ras point mutations with in vitro transformation potential is commonly found in cancers. Model-based analysis of these mutants uncovered a systems-level process that contributes to total Ras activation in cells. This predicted behavior was supported by experimental observations. We also used the model to identify a strategy in which a drug could cause stronger inhibition on the cancerous Ras network than on the wild-type network. This system-level analysis of the oncogenic Ras network provides new insights and potential therapeutic strategies.

Ras is a small guanosine triphosphatase (GTPase) that binds the guanine nucleotides guanosine triphosphate (GTP) and guanosine diphosphate (GDP) (*1*, *2*). Ras bound to GTP (Ras_{GTP}) is the "active" form with which downstream effector proteins spe-

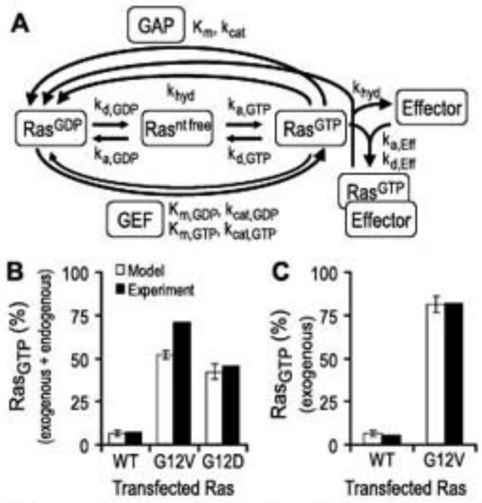


Fig. 1. The Ras GTPase signaling module and model validation. **(A)** We define the Ras GTPase signaling module to include Ras and the proteins with which it directly interacts (GEFs, GAPs, and Effectors). The model included all reactions of Ras with these proteins, as well as the reactions intrinsic to Ras (nt free refers to nucleotide-free). **(B)** Predicted steady-state percentage of GTP-bound total Ras in $Ras^{WT/WT}$ cells transfected with Ras^{WT}, Ras^{G12V}, or Ras^{G12D}. **(C)** Predicted steady-state percentage of exogenous Ras bound to GTP in $Ras^{WT/WT}$ cells transfected with Ras^{WT} or Ras^{G12V}. (B and C) Experimental data were from published studies (*12*–*14*). Model predictions are mean ± SD for nine different sets of protein concentration parameters.

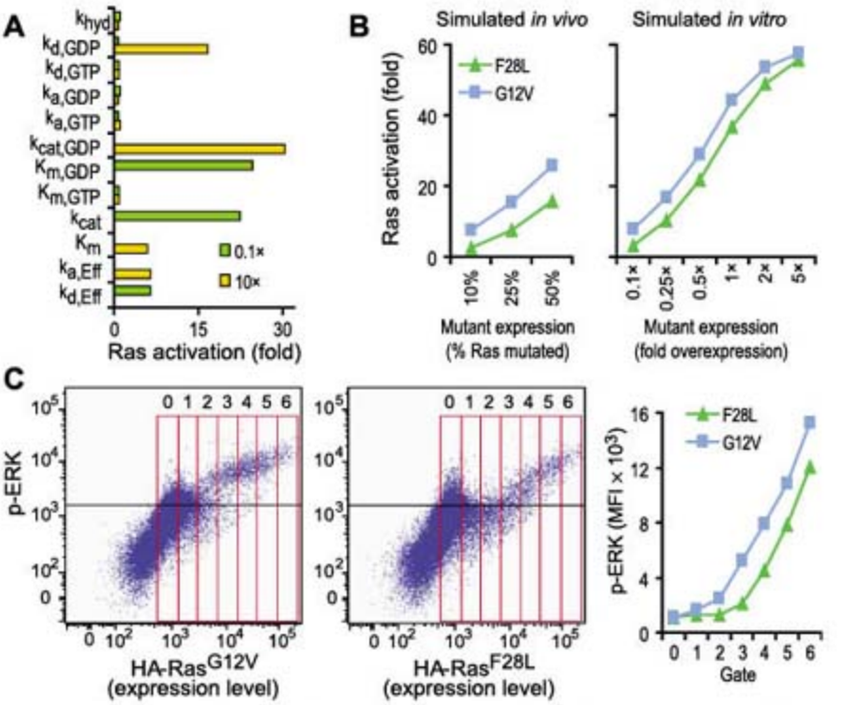


Fig. 2. A subset of Ras module properties can increase Ras signaling when altered. **(A)** Sensitivity analysis. The increase in Ras activation (total Ras-effector complex) due to an order of magnitude increase or decrease in each parameter of the Ras module. **(B)** Simulated in vivo and in vitro increases in Ras activation for fast-cycling (F28L) or GAP-insensitive (G12V) mutants. **(C)** (Left) Flow cytometry–based single-cell, quantitative assessment of Ras pathway activation (measured as phospho-ERK) as a function of expression levels of Ras^{F28L} or Ras^{G12V} in HEK-293T cells (50,000 events plotted for each). HA, hemagglutinin tagged. (Right) Plotting of the mean fluorescent intensity (MFI) for pERK versus mutant Ras protein expression levels, using the gates specified. These data are representative of two independent experiments. See fig. S1 for additional data under different transfection conditions.

cifically interact, thus propagating intracellular signals (*1*, *2*). Several downstream effector pathways are associated with cancer (*3*). Activating point mutations in the three isoforms of Ras are frequently found in human cancers (*4*). Although less than 5% of Ras is typically bound to GTP under basal resting conditions, over 50% of Ras is bound to GTP in cells with an activating Ras point mutation under the same conditions. High levels of unregulated Ras activation are thought to have a causal role in the development of cancer (*1*).

The activation state of Ras reflects a complex balance of several processes that coordinately regulate Ras_{GTP} (Fig. 1A) (*5*). Guanine nucleotide exchange factors (GEFs) facilitate dissociation and exchange of bound nucleotide from Ras. Ras inactivation (by hydrolysis of bound GTP to GDP) can be done at a slow rate by Ras itself through its intrinsic GTPase activity. GTPase-activating proteins (GAPs) increase the rate of GTP hydrolysis. Association of Ras_{GTP} with effector proteins can prevent regulatory enzymes from acting on Ras_{GTP} and can also prevent nucleotide dissociation, thus resulting in a sequestration of Ras_{GTP} (*6*). Attempts to understand Ras activation in a systems-level, cellular context must also consider concentrations of proteins that regulate Ras and the rate constants for their reactions.

We developed a mathematical model of the Ras signaling module using established methods for describing signal transduction networks (*7*), including heterotrimeric GTP-binding protein (G protein) and small GTPase signaling networks (*8*, *9*). The model accounts for GEF-catalyzed exchange, GAP-catalyzed hydrolysis, intrinsic association and dissociation of nucleotide from Ras, hydrolysis of GTP by Ras, and interaction of Ras_{GTP} with downstream effectors (*10*) (Fig. 1A). All rate constants needed to characterize these reactions have been measured previously for wild-type Ras (Ras^{WT}) (table S1). The magnitude of change has been measured for properties that differ largely from those of Ras^{WT} for several Ras mutants (table S2). The model requires 20 parameters to describe a module with Ras^{WT}. When both wild-type and mutant Ras are present, 14 additional parameters are required to incorporate the biochemical differences between them. Multiple sets of protein concentrations were considered to assess the robustness of our model because different cell types could express varying concentrations of module proteins [supporting online material (SOM) text]. Because oncogenic Ras point mutants result in increased steady-state concentrations of cellular Ras_{GTP} in the absence of stimulation, we focused on the steady-state behavior of Ras. We calculate two measures of Ras signaling output: the percentage of total Ras bound to GTP, and the concentration of Ras-effector complex formed (referred to here as "Ras activation"). When we discuss Ras activity, we refer to Ras signaling activity and not Ras GTPase activity. To test our model, we modeled a Ras^{WT} cell transfected with Ras^{WT}, Ras^{G12V}, or Ras^{G12D} [which indicate point mutations in which, for example, Gly^{12} is replaced by Val (G12V) (*11*)] to predict the percentage of total Ras (endogenous and exogenous), or exogenous Ras bound to GTP. Predictions were robust and matched well with experimental data (*12*–*14*) (Fig. 1, B and C, and table S3). Model predictions for the Ras GAP-deficient state associated with neurofibromatosis also support the ability of the model to make robust, quantitative predictions (SOM text).

[1]Beirne B. Carter Center for Immunology Research and the Department of Microbiology, University of Virginia, Charlottesville, VA 22908, USA. [2]Medical Scientist Training Program, University of Virginia, Charlottesville, VA 22908, USA. [3]Interdisciplinary Program of Biophysics, University of Virginia, Charlottesville, VA 22908, USA.

*To whom correspondence should be addressed. E-mail: Ravi@virginia.edu

Presumably, a mutation that disrupts any of the processes that regulate Ras could result in pathological Ras activation. However, only a few modes of deregulation are actually found in disease. We used our model to investigate the sensitivity of Ras activation to changes in each module property and found that Ras activation was largely affected by only four properties (Fig. 2A). There was good correlation between these four properties and the known physiological and pathological mechanisms of Ras pathway activation in many cancers (SOM text) (*2*). This analysis may help explain why only a limited number of the network properties result in pathological Ras activation when altered, i.e., network behavior is such that a disruption in a module property typically has a minimal effect on Ras activation.

Two of the properties that the model predicts will strongly influence Ras activation are altered in Ras point mutants with in vitro transformation potential: the rate of GDP dissociation from Ras ($k_{diss,GDP}$) is increased for fast-cycling mutants (e.g., Ras^{F28L}), and the k_{cat} for the GAP reaction is strongly decreased for GAP-insensitive mutants (e.g., Ras^{G12V}). Although both GAP-insensitive and fast-cycling Ras mutants have in vitro transformation potential (*15*, *16*), only GAP-insensitive Ras mutants are commonly found in cancers (*2*). When modeled at concentrations consistent with a spontaneous mutation in vivo, fast-cycling Ras^{F28L} showed approximately half the increase in forming Ras-effector complex of GAP-insensitive Ras^{G12V} (Fig. 2B). However, when we simulated concentrations consistent with the conditions of in vitro transformation assays, the difference between fast-cycling and GAP-insensitive mutants was reduced, and higher activation levels were achieved for both mutant classes (Fig. 2B). Results were similar for alternative sets of module protein concentrations (fig. S1).

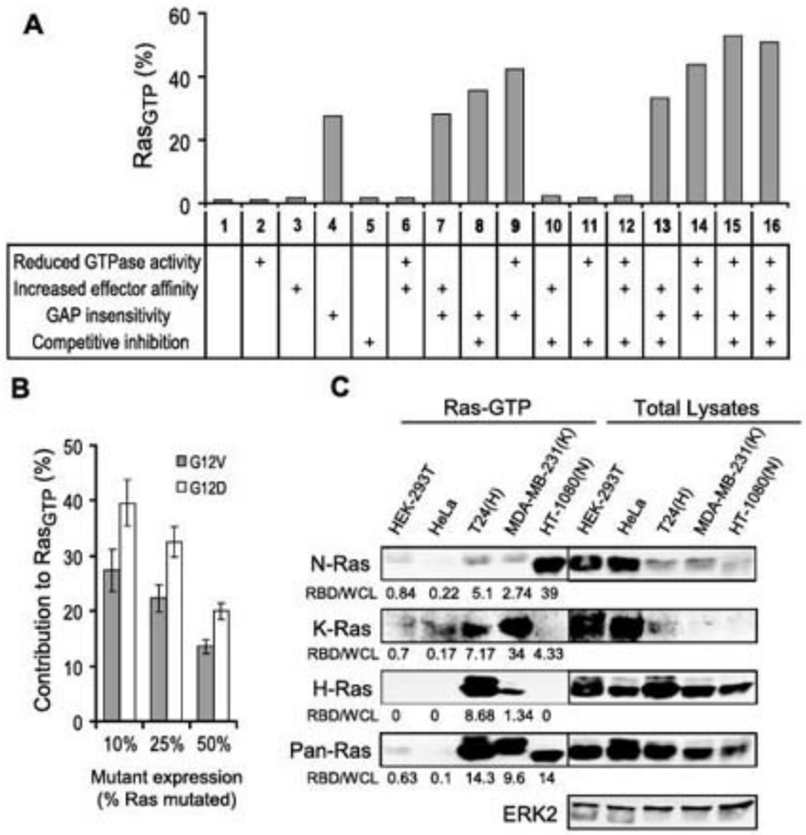


Fig. 3. Effects on Ras activation due to the multiple biochemical changes of the Ras^{G12V} point mutant. (**A**) Predicted percentage of GTP-bound total Ras for a $Ras^{G12V/WT}$ module when the altered biochemical properties of the Ras^{G12V} mutant are considered as individual entities or in combination. (**B**) Relative contribution of competitive inhibition of Ras GAPs by Ras^{G12V} or Ras^{G12D} to the total increase in Ras_{GTP} formed for different percentages of total Ras mutated. Results presented are means ± SD for the nine different sets of protein concentrations. (**C**) Proportion of total Ras as Ras_{GTP} in cell lines with GAP-insensitive Ras mutants (T24, MDA-MB-231, and HT1080) and in cell lines without a known Ras mutation (HEK-293T and HeLa), measured by precipitation of lysates using the Ras_{GTP}-binding domain of Raf (RBD). To determine the fraction of a given isoform bound to GTP, immunoblotting was done using isoform-specific antibodies or a pan-Ras antibody. The signals for Ras_{GTP} in the different cells lines were normalized against total cellular Ras in the same cell line. Results reflect two to five independent experiments, and the differences were significant ($P < 0.05$, Student's t test).

To test these predictions experimentally, we used flow cytometry to obtain quantitative, single-cell measurements of active Ras as a function of mutant expression level for either GAP-insensitive Ras^{G12V} or fast-cycling Ras^{F28L}. The amount of activated, phosphorylated extracellular signal–regulated kinase (pERK) was used as a readout of Ras activation (*2*). Expression of small amounts of Ras^{F28L} caused production of less pERK than did similar amounts of Ras^{G12V} (Fig. 2C and fig. S2), in agreement with our model (Fig. 2B). Both mutants caused large amounts of pERK at higher expression levels (Fig. 2C). Thus, our computational and experimental results suggest that fast-cycling Ras point mutants may not be found in cancers because they cause a smaller increase in Ras signal amplitude than GAP-insensitive mutants for the concentration range likely to occur with spontaneous mutations.

Biochemical measurements have identified three differences between Ras^{WT} and Ras^{G12V} that might contribute to increased Ras_{GTP}: (i) The rate of GTP hydrolysis for Ras^{G12V} seems unaffected by the addition of GAP (GAP insensitivity) (*17*); (ii) the intrinsic GTPase activity of Ras^{G12V} is approximately one order of magnitude slower than that of Ras^{WT} (reduced intrinsic GTPase activity) (*18*); and (iii) the affinity of Ras^{G12V} for its downstream effector Raf is approximately doubled (increased effector affinity) (*19*). GAP insensitivity has been proposed as the primary cause of increased Ras activation (*1*). However, this hypothesis has not been testable experimentally because Ras^{G12V} exhibits all of these altered biochemical properties simultaneously.

In our model, GAP insensitivity alone increased the percentage of total Ras existing as Ras_{GTP} to about half that predicted when all mutant properties were included (Fig. 3A and table S4). In contrast, reduced intrinsic GTPase activity and increased effector affinity individually had minimal predicted effect on Ras activation (Fig. 3A; table S4). Reduced intrinsic GTPase activity did cause a predicted further increase when combined with GAP insensitivity, and increased effector affinity also caused a small increase when combined with GAP insensitivity (Fig. 3A). Essentially similar results were obtained when parameters for Ras^{G12D} were used, which suggests that these results are applicable to other GAP-insensitive Ras point mutants (table S5).

The combination of GAP insensitivity, reduced GTPase activity, and increased effector affinity did not account for all of the increased Ras activation. Although Ras^{G12V} is GAP-insensitive, it associates with GAP proteins (*20*), and could competitively inhibit Ras GAP activity on Ras^{WT}. In our model, formation of Ras_{GTP} was increased when competitive inhi-

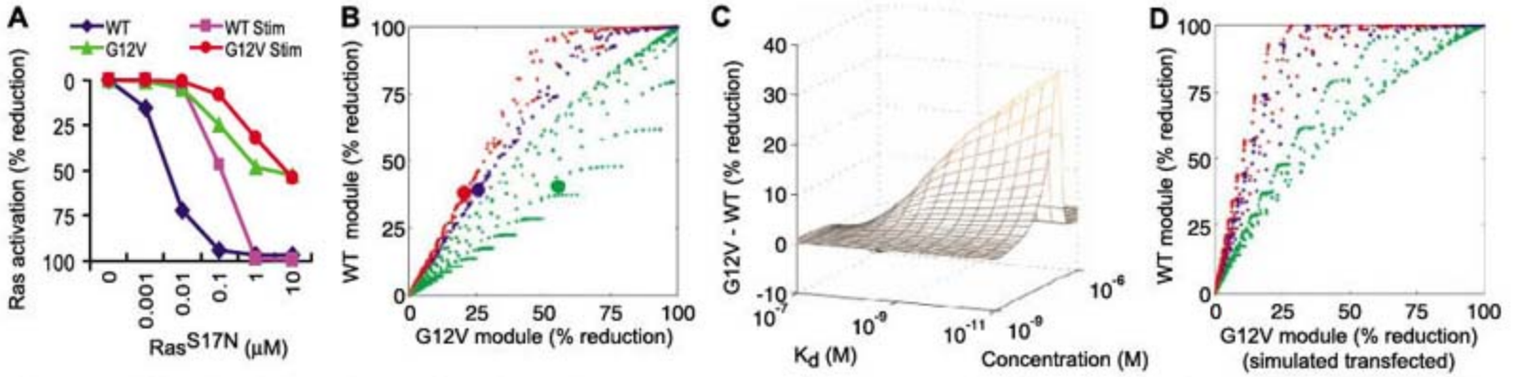


Fig. 4. Model-based analysis of pharmacological strategies to preferentially inhibit the cancerous Ras module. **(A)** Dose-dependent decrease in Ras activation (concentration of Ras-effector complex) caused by dominant-negative Ras^{S17N} for wild-type Ras networks expressing exogenous Ras^{WT} or Ras^{G12V} and in the presence or absence of stimulation. **(B)** Predicted reduction in Ras activation for the different drug strategies on the $Ras^{G12V/WT}$ module and on the stimulated $Ras^{WT/WT}$ module. Each point corresponds to one of 600 total conditions (15 different affinities and 40 different concentrations) for each of the three strategies. Drug A, red; drug B, green; drug C, blue. Large filled circles represent effects for relevant drug-Ras interactions at $K_d = 1$ nM and drug concentration at 0.15 μM. **(C)** The difference in percent reduction between oncogenic G12V module inhibition and wild-type module inhibition for drug B, presented as a function of drug affinity and concentration. **(D)** Same as (B), but the G12V module is now modeled as a $Ras^{WT/WT}$ cell transfected with exogenous Ras^{G12V}.

bition of Ras GAP was combined with GAP insensitivity (Fig. 3A). This increase did not result from changes in the fraction of Ras^{G12V} that was GTP-bound (~85% in both cases), rather it was due to an increase in the GTP-bound fraction of Ras^{WT} (from 1 to 15%) (table S4). Results were similar when alternative sets of module protein concentrations were modeled (fig. S3). This analysis has been done with the assumption that 50% of total cellular Ras is mutated. As there are three isoforms, we performed the same analysis for cases when less than 50% of total cellular Ras is mutated and obtained similar results (tables S6, S7). The relative contribution of competitive inhibition on Ras activation increased when mutated Ras made up a smaller fraction of total Ras in the cell (Fig. 3B). This further suggests competitive inhibition is likely to occur after point mutation in one of the *ras* genes in the cell.

To test the predicted effect of competitive inhibition of Ras GAPs on Ras activation we performed a Ras-binding domain (RBD) pull-down assay on three cancer cell lines that harbor GAP-insensitive Ras mutations (T24: H-Ras^{G12V}; HT1080: N-Ras^{Q61L}; MDA-MB-231: K-Ras^{G13D}) and, as controls, two cell lines in which activating Ras mutations have not been described (HeLa, HEK-293T). We found a statistically significant increase in the proportion of GTP-bound Ras^{WT} for the cells harboring GAP-insensitive Ras point mutants (Fig. 3C). Taken together, our combined computational and experimental work uncovered a systems-level process that our simulations predict could contribute upwards of 30% of the Ras_{GTP} found in a cell with an activating point mutation. Our results indicate that cancers will likely display significant heterogeneity because of secondary activation of other WT Ras isoforms in the same cell.

Ideally, a therapeutic strategy to inhibit Ras signaling would have a much stronger effect on diseased cells with an oncogenic Ras mutation than on healthy cells without a Ras mutation (*2*). The Ras signaling network is characterized by different properties for cancerous and non-cancerous cells, and a nonlinear dynamical system can have quantitatively and/or qualitatively different behaviors for different sets of system parameters. We therefore hypothesized that a drug binding both Ras^{G12V} and Ras^{WT} with equal affinity could block Ras signaling in the cancerous network more than the wild-type network. To first test the ability of our model to assess possible pharmaceutical strategies, we compared the dose-dependent inhibition of Ras activation by dominant-negative Ras on a network expressing exogenous Ras^{G12V} or Ras^{WT}. The model showed that dominant-negative Ras^{S17N} had a stronger effect on the wild-type network (Fig. 4A and fig. S4). This is consistent with experimental observations that Ras^{S17N} inhibited Ras^{WT} signaling more than it did oncogenic Ras signaling (*21*). This observation, once considered "unexpected" (*21*), is readily explained from our systems-level model.

To search for a therapeutically beneficial strategy, we extended our model to examine three possible strategies of drug intervention: (i) a hypothetical drug A that binds and sequesters Ras_{GDP}, (ii) a potential drug B that binds and sequesters Ras_{GTP}, and (iii) a drug C that binds and sequesters both Ras_{GDP} and Ras_{GTP} equally well (fig. S5). We ran simulations of the extended model to test each drug strategy. Only drug B caused a greater reduction in Ras-effector interactions in the cancerous network ($Ras^{G12V/WT}$) than in the wild-type network ($Ras^{WT/WT}$). In contrast, drug A and drug C caused a greater reduction in Ras-effector interactions for the $Ras^{WT/WT}$ signaling module than for the $Ras^{G12V/WT}$ module (Fig. 4B). Drug B was predicted to have this behavior for a wide range of conditions (Fig. 4C). Results were similar when alternative sets of module protein concentrations were modeled (fig. S6). Previous experimental studies of potential Ras inhibitors have used $Ras^{WT/WT}$ cells that had been transfected with Ras^{G12V} (*22*, *23*). In our simulations, drug B was no longer selective under these conditions (Fig. 4D). Thus, the use of Ras^{G12V}-transfected cell lines may yield false-negatives when used to screen for drugs that selectively target cancerous cells.

Oncogenic Ras has been well studied at the genetic, biochemical, and whole-animal levels. This molecular network level analysis of the Ras signaling module provided a bridge between the biochemical data at the protein level and Ras activation at the cellular level. The systems-level analysis of the wild-type Ras network and an oncogenic Ras network also identified a strategy that selectively targeted the mutant network as a result of quantitative differences between the two networks. Similar strategies might be found for other networks where mutations alter biochemical properties and result in pathological deregulation. These results highlight the promise of systems-level mathematical models to study signaling pathways altered in disease and to identify potential drug strategies.

References and Notes

1. M. Malumbres, M. Barbacid, *Nat. Rev. Cancer* **3**, 459 (2003).
2. J. Downward, *Nat. Rev. Cancer* **3**, 11 (2003).
3. G. A. Repasky, E. J. Chenette, C. J. Der, *Trends Cell Biol.* **14**, 639 (2004).
4. J. L. Bos, *Cancer Res.* **49**, 4682 (1989).
5. M. S. Boguski, F. McCormick, *Nature* **366**, 643 (1993).
6. C. Herrmann, G. Horn, M. Spaargaren, A. Wittinghofer, *J. Biol. Chem.* **271**, 6794 (1996).

7. B. B. Aldridge, J. M. Burke, D. A. Lauffenburger, P. K. Sorger, *Nat. Cell Biol.* **8**, 1195 (2006).
8. N. I. Markevich *et al.*, *Syst. Biol. (Stevenage)* **1**, 104 (2004).
9. S. J. Bornheimer, M. R. Maurya, M. G. Farquhar, S. Subramaniam, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 15899 (2004).
10. Materials and methods are available as supporting material on *Science* Online.
11. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
12. G. Bollag *et al.*, *J. Biol. Chem.* **271**, 32491 (1996).
13. J. B. Gibbs, M. S. Marshall, E. M. Scolnick, R. A. Dixon, U. S. Vogel, *J. Biol. Chem.* **265**, 20437 (1990).
14. S. Boykevisch *et al.*, *Curr. Biol.* **16**, 2173 (2006).
15. L. A. Feig, G. M. Cooper, *Mol. Cell. Biol.* **8**, 2472 (1988).
16. G. Patel, M. J. MacDonald, R. Khosravi-Far, M. M. Hisaka, C. J. Der, *Oncogene* **7**, 283 (1992).
17. M. R. Ahmadian, U. Hoffmann, R. S. Goody, A. Wittinghofer, *Biochemistry* **36**, 4535 (1997).
18. J. F. Eccleston, K. J. Moore, G. G. Brownbridge, M. R. Webb, P. N. Lowe, *Biochem. Soc. Trans.* **19**, 432 (1991).
19. E. Chuang *et al.*, *Mol. Cell. Biol.* **14**, 5318 (1994).
20. U. S. Vogel *et al.*, *Nature* **335**, 90 (1988).
21. D. W. Stacey, L. A. Feig, J. B. Gibbs, *Mol. Cell. Biol.* **11**, 4053 (1991).
22. N. E. Kohl *et al.*, *Science* **260**, 1934 (1993).
23. G. L. James *et al.*, *Science* **260**, 1937 (1993).
24. We are grateful to S. Walk for the HA-Ras plasmids, and the members of the Ravichandran lab for helpful discussions. E.S. was supported through a cancer training grant (NCI), and Z.M. was supported by a Biodefense training grant (NIAID). This work was supported in part by a grant from the NIGMS (to K.S.R.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5849/463/DC1
Materials and Methods
SOM text
Figs. S1 to S7
Tables S1 to S7
References

4 May 2007; accepted 6 September 2007
10.1126/science.1144642

Light-Responsive Cryptochromes from a Simple Multicellular Animal, the Coral *Acropora millepora*

O. Levy,[1] L. Appelbaum,[2] W. Leggat,[1] Y. Gothlif,[3] D. C. Hayward,[4,6] D. J. Miller,[5,6] O. Hoegh-Guldberg[1,5]

Hundreds of species of reef-building corals spawn synchronously over a few nights each year, and moonlight regulates this spawning event. However, the molecular elements underpinning the detection of moonlight remain unknown. Here we report the presence of an ancient family of blue-light–sensing photoreceptors, cryptochromes, in the reef-building coral *Acropora millepora*. In addition to being cryptochrome genes from one of the earliest-diverging eumetazoan phyla, *cry1* and *cry2* were expressed preferentially in light. Consistent with potential roles in the synchronization of fundamentally important behaviors such as mass spawning, *cry2* expression increased on full moon nights versus new moon nights. Our results demonstrate phylogenetically broad roles of these ancient circadian clock–related molecules in the animal kingdom.

Many organisms possess endogenous clocks that respond to rhythmic changes in light and temperature caused by Earth's rotation (*1*, *2*), allowing them to anticipate daily and annual environmental cycles and to adjust their biochemical, physiological, and behavioral processes accordingly (*1*). The circadian clock uses cues such as light to entrain endogenous oscillators, which in turn control rhythmic outputs of a wide range of organisms (*2*). Even simple animals such as medusae (scyphozoan or hydrozoan cnidarians) have specialized light-sensing organs known as ocelli (eyes) or eyespots. These photoreceptors react to changes in light intensity and are responsible for phototaxis and other behavioral responses to light (*3*). However, anthozoan cnidarians (corals, sea anemones, and sea pens) lack specialized sense organs yet display photosensitive behavior (*4*–*11*). The synchronized mass spawning on the Great Barrier Reef (GBR) in Australia is a spectacular example of the photosensitive responses exhibited by these organisms (*7*–*9*). Over several nights after the full moon in late spring each year, hundreds of coral species spawn en masse, with the final trigger being changes in the lunar irradiance intensity (*8*, *11*).

The specific cellular mechanisms involved in light detection by reef-building corals (Anthozoa, Cnidaria) have remained elusive. Biophysical data (*4*) show that corals are highly sensitive to blue light, which is also known to entrain the circadian clocks of insects and mammals (*12*) via cryptochromes (CRYs), which are DNA photolyase–like photoreceptor proteins. The roles of cryptochromes differ subtly between mammals and insects; the proteins function as circadian oscillator components in *Mus* but as photoreceptors for clock entrainment in *Drosophila* (*13*, *14*). To date, CRYs have been identified only in higher animals such as vertebrates and insects, although related (and divergent) proteins have been reported in plants and eubacteria (*12*).

We used degenerate primers based on sequences conserved between *Mus*, *Drosophila*, *Xenopus*, and *Danio* to clone two *cry* genes from the coral *Acropora millepora* (*15*). The proteins encoded by the genes *cry1* and *cry2* each contain an N-terminal photolyase-related region (PHR) bearing two chromophore-binding domains and C-terminal domains extending 54 (CRY1) or 27 (CRY2) amino acids in length. *cry1* and *cry2* were also identified in expressed sequence tag (EST) data sets generated from *A. millepora* larvae (*16*), as were two additional genes known here as *cry3* (*15*) and *cry-dash*. Because the cDNA library was constructed from aposymbiotic larvae, these *cry* genes are likely to be from coral rather than from associated symbiotic algae or marine microbes.

Phylogenetic analyses (Fig. 1) emphasize the similarity of coral CRYs and their vertebrate counterparts. Coral CRY1 belongs to the mammalian-type (m-type) CRY group. Both CRY1 and CRY2 are only distantly related to the *Drosophila*-type CRYs. CRY2 more closely resembles the *Danio* photoreceptor candidate CRY4-type (*17*) and is basal to the clade comprising both the m-type CRYs and the (6-4) photolyases. Coral CRY-DASH is a typical CRY-DASH protein (*18*) and is basal in the animal CRY-DASH clade. This analysis suggests that coral CRYs may represent ancestral members of the protein family in the animal kingdom, potentially providing insights into the origins of light perception in animals.

On the GBR, we investigated (*15*) whether the expression of *cry1* and *cry2* in corals is rhythmic only under light/dark (LD) cycles as in *Drosophila* (*13*) or driven by an endogenous

[1]Centre for Marine Studies, University of Queensland, St. Lucia 4072 QLD, Australia. [2]Center for Narcolepsy and Howard Hughes Medical Institute, Stanford University, Palo Alto, CA, USA. [3]Department of Neurobiochemistry, Faculty of Life Sciences, Tel Aviv University, Israel. [4]Research School of Biological Sciences, Australian National University, Canberra ACT 2601, Australia. [5]Australian Research Council (ARC) Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, and University of Queensland, St. Lucia 4072 QLD, Australia. [6]ARC Centre for the Molecular Genetics of Development, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia.

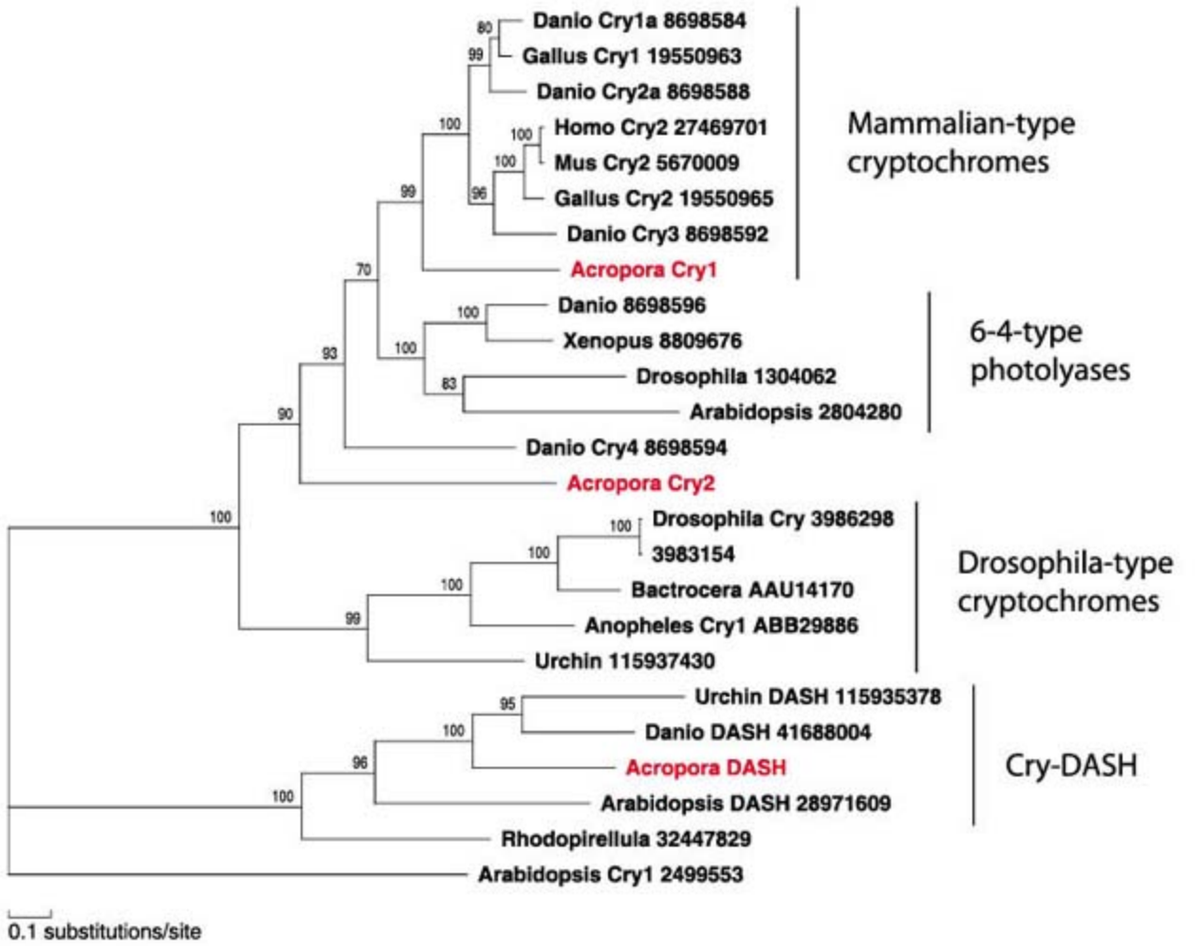


Fig. 1. Phylogenetic relationships of the coral CRY and photolyase proteins resulting from maximum likelihood analysis of a representative range of CRY and photolyase proteins. Sequences analyzed were retrieved from public electronic databases. The accession numbers of each protein are given together with the genus name. Numbers on nodes represent percentages of 1000 bootstrap replicates, reflecting support for the topology shown.

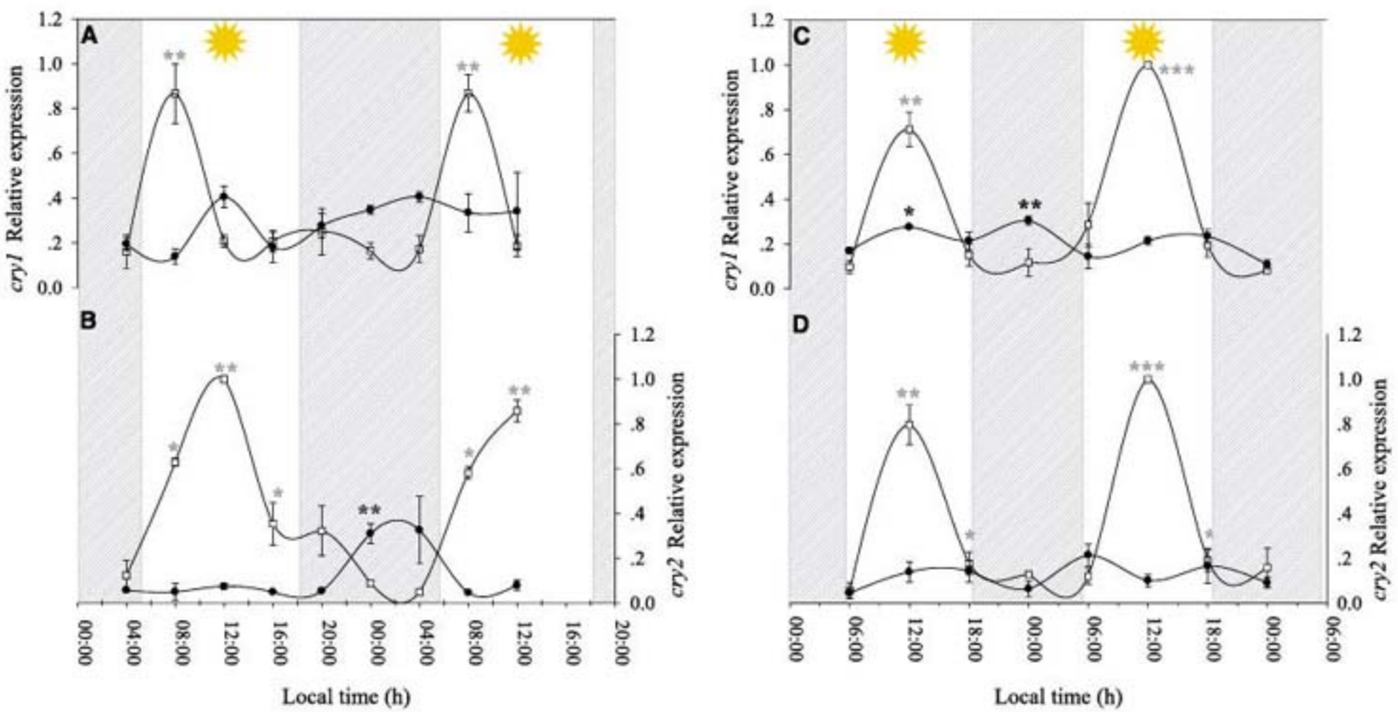


Fig. 2. Temporal expression patterns of *cry1* and *cry2* in *A. millepora* under LD (white squares) and DD (black circles) cycles analyzed with quantitative polymerase chain reaction. (**A** and **B**) A 32-hour cycle with sampling intervals of 4 hours (A). Quantitative analysis of *cry1* revealed a significant effect of LD ($P = 0.035$), as well as a significant effect of sampling time (time, $P < 0.001$). (B) Expression of *cry2* (LD, $P = 0.026$; time, $P < 0.001$). (**C** and **D**) a 42-hour cycle with sampling intervals of 6 hours. (C) Expression of *cry1* (LD, $P = 0.016$; time, $P < 0.001$). (D) *Cry2* expression (LD, $P = 0.013$; time, $P < 0.001$). There was no significant effect of sampling time X cycle ($P > 0.05$ for both *cry1* and *cry2* under 42-hour cycle treatment), followed by ANOVA with repeated measures. Each value was normalized to β-actin and converted to a percentage of the maximal level for each gene. Values (mean ± SE) were tested by ANOVA with a linear contrast method within groups in order to distinguish between the LD/DD rhythm amplitude of *cry1* and *cry2*. For *Cry1* DD, $P > 0.01$; *cry1* LD, $P < 0.01$; *cry2* DD, $P > 0.05$; *cry2* LD, $P < 0.01$. Gray areas represent ambient darkness, and time points with asterisks are significantly different (gray asterisks, LD; black asterisks, DD). * represents $P < 0.05$, ** represents $P < 0.01$, and *** represents $P < 0.001$. Sample size = 3 colonies.

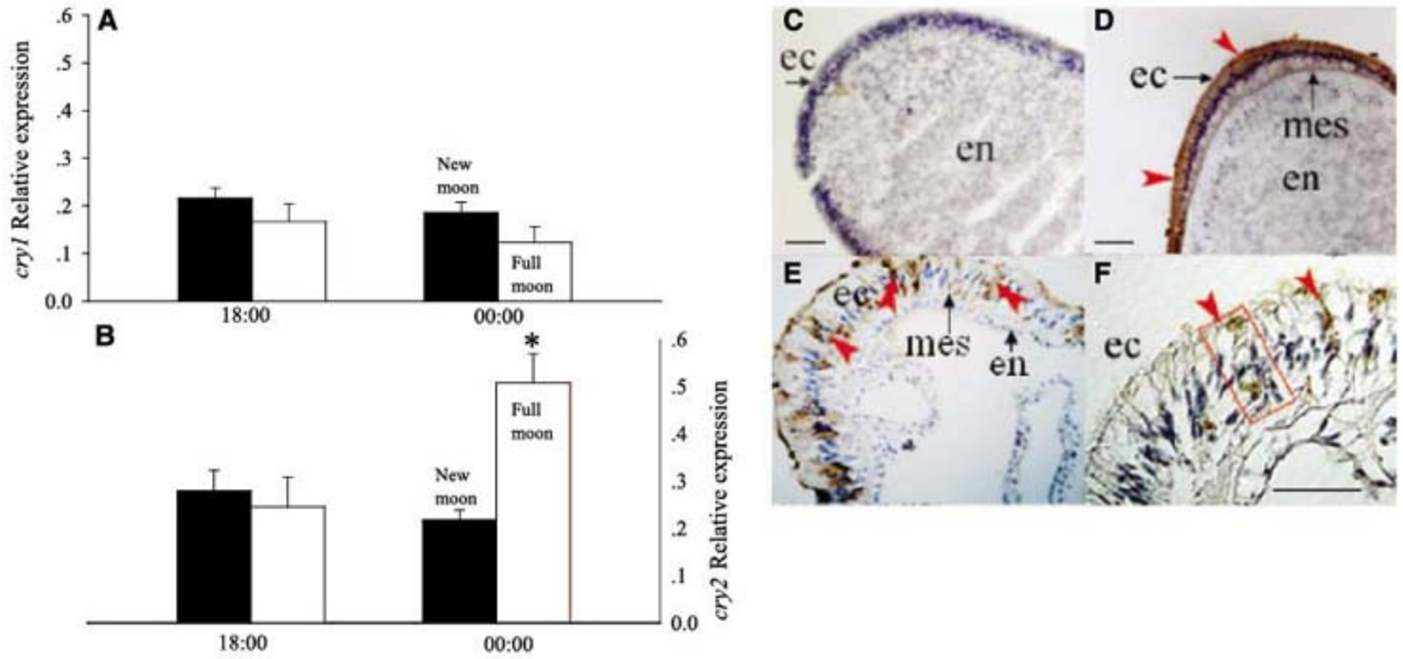


Fig. 3. (**A** and **B**). Quantitative analysis of *cry1* and *cry2* in the 2 consecutive months of August and September 2005, comparing new moon nights to full moon nights at time points of 18:00 and 00:00. Each value is the average time point of the 2 months of sampling (mean ± SE). ANOVA RM, $P > 0.05$ for *cry1*, $^*P < 0.05$ for *cry2*; sample size = 4 colonies. (**C** to **F**). Localization of *cry1* and *cry2* in the coral host tissue. Immunohistochemistry was performed with specific antibodies against CRY1 and CRY 2 in 72-hour larvae and in adult colonies of *A. millepora*. (C) Control. Larvae probed with preimmune serum were free of signals. (D) CRY2 is expressed (brown staining indicates the antibody-specific staining) in the ectoderm layer of the larval tissue. (E) and (F) Protein localization in the ectoderm layer of adult *A. millepora* corals when treated with specific antibodies against CRY1 (E) and CRY2 (F) (brown). ec, ectoderm; en, endoderm; mes, mesogloea. Scale bars, 50 μm. Red arrowheads mark expression of the antibody in (D) to (F); the rectangle shows one cell in the ectoderm layer.

oscillator as in mammals (*19*). The coral genes exhibited daily rhythms under LD cycles, with peak expression at zeitgeber (time-giver, ZT) 3 for *cry1* (ZT = 0 corresponding to first nautical light, which was 05:00 local time) and ZT 7 for coral *cry2* [LD expression ratios were as follows: *cry1*, 5.4; *cry2*, 8.1 (Fig. 2, A and B)]. *Cry1* mRNA levels were arrhythmic under continued darkness (DD), [analysis of variance (ANOVA) repeated measures (RM) $P > 0.05$], whereas *cry2* expression changed significantly ($P < 0.05$) only at ZT 17 ($P = 0.01$) (Fig. 2, A and B). Experiments carried out 4 months later (under LD and DD conditions over 2 consecutive days) confirmed that the expression of both *cry1* and *cry2* is daylight-dependent, with significantly higher transcript levels under LD at ZT 6 (ANOVA RM $P < 0.01$). Under DD, *cry1* expression exhibited low amplitude with significantly higher mRNA levels at 12:00 and 00:00 ($P < 0.05$), whereas *cry2* expression did not significantly change under DD conditions (Fig. 2, C and D). The aberrant timing of expression under DD versus LD indicates a decay of rhythm in the absence of a ZT, suggesting that rhythmic expression of coral cryptochromes is not driven by an endogenous oscillator (*20*). Both genes exhibited a rhythmic expression pattern under LD, but with a different phase, suggesting that expression might be controlled by different transcriptional regulatory mechanisms within the same tissue environment.

To test the involvement of *cry* genes in sensing and responding to moonlight, fragments from each of four colonies on the reef flat were sampled four times a day during the full and new moon phases of August and September 2005 (data are presented for 18:00 and 00:00 hours). Additionally, we sampled colonies on a full moon night in November 2005 5 days before the mass spawning event (fig. S1).

During full and new moon nights, *cry1* showed similar transcript levels during August and September 2005 at 18:00 (ANOVA RM, $P > 0.05$) (Fig. 3A) (*15*). In contrast, coral *cry2* showed significantly higher expression at midnight under full than new moonlight when compared at 18:00 ($P < 0.05$) (Fig. 3B). The expression pattern associated with *cry2* suggests that it may entrain the intrinsic clock of corals to the lunar phase; however, roles for other photopigments such as opsins cannot currently be ruled out.

Consistent with roles as photoreceptor proteins, immunohistochemistry revealed that both CRY1 and CRY2 proteins (*15*) are restricted to the ectoderm in both larval and adult corals (Fig. 3, D to F, and fig. S2B; control being negative, Fig. 3C and fig. S2A). The corresponding mRNAs showed the same patterns in in situ hybridization experiments (*15*, *21*) (fig. S2, C and D).

Our discovery of cryptochromes in reef-building corals reveals that the basic mechanisms by which insects and mammal circadian oscillators respond to light were in place at the origins of multicellularity in animals. The presence of CRYs in a phylum close to the base from which all multicellular animals diverge supports the hypothesis that these proteins evolved under the blue light of the Precambrian ocean, possibly as a means to avoid high daytime ultraviolet levels near the surface (*22*). The expression patterns of coral CRYs in response to daylight (*cry1* and *cry2*) and moonlight (*cry2*) also suggest that cryptochromes may mediate the spectacular mass spawning event of invertebrates (*8*, *9*), adding an evolutionary dimension to circadian clock biology.

References and Notes

1. C. S. Pittendrigh, *Annu. Rev. Physiol.* **55**, 17 (1993).
2. S. Panda, J. B. Hogenesch, S. A. Kay, *Nature* **417**, 329 (2002).
3. V. J. Martin, *Can. J. Zool.* **80**, 1703 (2002).
4. M. Y. Gorbunov, P. G. Falkowski, *Limnol. Oceanogr.* **47**, 309 (2002).
5. O. Levy, Z. Dubinsky, Y. Achituv, *J. Exp. Biol.* **206**, 4041 (2003).
6. O. Levy, Y. Achituv, Y. Z. Yacobi, N. Stambler, Z. Dubinski, *J. Exp. Mar. Biol. Ecol.* **328**, 35 (2006).
7. S. M. Marshall, T. A. Stephenson, *Nat. Hist.* **3**, 219 (1933).
8. P. L Harrison *et al.*, *Science* **223**, 1187 (1984).
9. R. C. Babcock *et al.*, *Mar. Biol.* **90**, 379 (1986).
10. R. C. Babcock, B. L. Shallis, C. J. Simpson, *Coral Reefs* **13**, 161 (1994).
11. P. J. Jokiel, R. Y. Ito, P. M. Liu, *Mar. Biol.* **88**, 167 (1985).

FROM MORGAN TO MICROARRAYS:

GENE MAPPING HITS THE BIG TIME

SNPs, single-base mutations scattered throughout the genome, can allow geneticists to map traits with astonishing resolution and speed. An overview of some of the new technologies that companies are offering for SNP profiling, and the diverse applications for which researchers are using them, reveals a rapidly evolving field with a promising future. **By Alan Dove**

In 1910, Thomas Morgan observed something unusual in one of the many jars in his Columbia University laboratory: a white-eyed fruit fly. It was one of the best things to happen to biology since Charles Darwin landed a berth aboard *HMS Beagle*. Soon determining that the eye color trait was linked to the sex of the fly, Morgan embarked on a series of experiments that jump-started modern genetics. Biologists have been mapping genes with increasingly sophisticated tools ever since.

Later 20th-century researchers eventually discovered microsatellites, short DNA sequences that had been duplicated throughout the genomes of eukaryotes during evolution, providing more precise genetic signposts than Morgan's phenotype-based maps. While many researchers still use this approach (see "The Decaying Orbit of Microsatellites"), the era of genome sequencing has brought another tool to the fore: single nucleotide polymorphisms, or SNPs.

The Numbers Game

SNP mapping has quickly become the standard tactic for two types of genetic studies in humans: linkage analysis and genomewide association. Linkage analysis is the direct descendant of Morgan's original experiments. By tracking the inheritance of a trait and a set of genetic signposts, such as microsatellites or SNPs, through several generations of a family, researchers can map the trait to specific chromosomal regions.

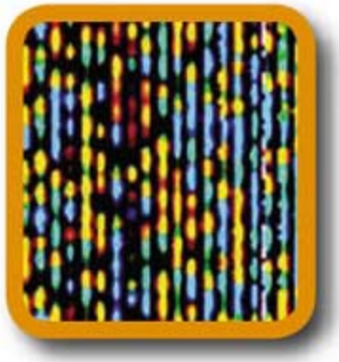
Genomewide association (GWA), also called "genetics without families," treats the entire population as a single giant family, then tries to find associations between specific traits and sets of SNP markers. GWA eliminates the need to find large families with accurate, multigenerational medical records, and also allows geneticists to probe common, complex diseases. Unsurprisingly, it has become an extremely popular technique.

"Within the last seven months or so, we have had 14 studies come out doing [GWA] showing significant findings in various diseases, including prostate cancer, type 2 diabetes, [inflammatory bowel disease], and Crohn's disease," says Todd Dickinson, director of product marketing at **Illumina**, based in San Diego, California.

Because they sample completely outbred populations, GWA studies require a different approach from traditional linkage analyses. In GWA, researchers generally screen a relatively small population using hundreds of thousands of SNPs, to highlight the regions of the genome most likely involved in a disease. In a second phase, the focus shifts to just those regions, using smaller numbers of SNPs across a much larger population, to provide statistical power.

In the first phase of a GWA study, screening more SNPs increases the chance of finding a meaningful association, so Illumina and their principal rival in the SNP market, **Affymetrix**, have been racing to boost the densities of its array-based assay systems. "We have evolved from 10,000 to 100,000 to 500,000, and our latest array actually has over 900,000 SNPs on it," says Jessica Tonani, a genotyping specialist at the Santa Clara, California, headquarters of Affymetrix.

Besides boosting the sheer number of SNPs on arrays, manufacturers are also trying to make them more informative. "We have intentionally selected to target what we call tag SNPs. These are SNPs that represent a group of SNPs that tend to be inherited together," says Dickinson. Illumina asserts that this effectively multiplies the screening power of its mil- continued »



Genomewide association, also called "genetics without families," treats the entire population as a single giant family.

CREDIT: (MIDDLE) PAUL REYNOLDS

Look for these **Upcoming** Articles
RNAi: Therapeutic Applications— November 2
Protein-Protein Interactions — November 30

Inclusion of companies in this article does not indicate endorsement by either AAAS or Science, *nor is it meant to imply that their products or services are superior to those of other companies.*

"We're hitting a point of kind of diminishing returns when you look at actually adding increased SNP content."

lion-SNP chip.

The drive for more density may be reaching a practical plateau, though. "We're hitting a point of kind of diminishing returns when you look at actually adding increased SNP content," says Tonani, who adds that most GWA studies would now benefit more from studying larger patient populations than from screening more SNPs. To enable that, she says Affymetrix is now focusing on lowering the prices of its high-density SNP arrays rather than packing more SNPs into them.

When Less Is More

Researchers working on the second phase of a GWA study, or those doing traditional family-based linkage analyses, have different SNP profiling needs. Rather than screening thousands of SNPs in dozens of individuals, they are screening dozens of SNPs in thousands of individuals.

"Typically for the whole genome analysis, people will identify a candidate region that they're interested in, or several genes, and need to move to a lower number of SNPs but a higher number of samples," says Phoebe White, senior director of genotyping at **Applied Biosystems (ABI)** in Foster City, California.

To cater to that market, ABI now offers the SNPlex platform, which combines real-time PCR with the company's ubiquitous capillary electrophoresis platform to track 12 to 48 SNPs per experiment. Because each SNPlex reaction is much cheaper than a high-density array, even small labs can screen SNPs across thousands of samples. "A large majority of labs these days do have a [capillary electrophoresis] platform, and so what we wanted to do was enable them to do these kinds of genotyping studies without bringing in a new type of platform," says White.

While using equipment on hand is obviously cheaper, labs that frequently plan to check specific genotypes may want to investigate the new LightCycler 480 from **Roche Diagnostics** in Basel, Switzerland. Besides functioning as a high throughput quantitative PCR platform, which can carry out a 40-cycle reaction on a 384-well plate in just 40 minutes, the LightCycler can also process SNP genotypes with a unique DNA melting point assay. Rather than designing separate primers for each allele of each SNP and carrying out complex enzymatic reactions, researchers simply create one set of primers per SNP and perform a single physical assay, in which the precise primer-template melting point reveals the sequences of the template's SNP alleles. Any mismatches between a SNP allele and the primer sequence will change the melting point, and the LightCycler's unbiased algorithm can even identify previously unknown SNP alleles.

For specific gene mapping applications, investigators may narrow their focus even further, looking at specific candidate genes associated with just a handful of SNPs. For example, patient responses to the anticoagulant warfarin associate tightly with a single gene. "It's critical now to understand what your genotype is to know what kind of dosage you need, but the number of SNPs you need to screen for is less than 20," says White. At that scale, even the coverage provided by the SNPlex kit may be too broad, so researchers resort to profiling individual SNPs by standard real-time PCR.

While screening fewer SNPs is undeniably cheaper than screening many, groups working on GWA must be careful not to simplify the assay prematurely. "If you're to move forward with a subset of SNPs, oftentimes you would miss certain things," says Affymetrix's Tonani. Pointing to the recent GWA mapping of potential diabetes-related genes, Tonani adds that "in three separate studies, and of the nine genes that they actually identified, eight of the nine genes wouldn't have been identified for this subset panel in at least one of the studies."

Into the Core

Like most new technologies, SNP profiling systems have become progressively cheaper in recent years. Indeed, according to some estimates, the cost per SNP genotype has dropped more than a thousandfold since 2000. Nonetheless, with GWA studies now profiling close to a million SNPs per patient, large-scale genotyping remains an expensive undertaking.

That's why most labs now send their big SNP projects to a dedicated core facility. "I think nobody is buying the system and setting it up for a specific project. It's always some kind of facility, either a company or within a place like ours," says Ann-Christine continued »

The Decaying Orbit of Microsatellites

Before the advent of single nucleotide polymorphism (SNP) profiling, gene mapping studies usually involved tracking microsatellite markers—small, frequently repeated DNA sequences—with gels. Lots and lots of gels. By identifying microsatellites that cosegregated with a particular trait, researchers could narrow the search for the responsible gene, first to a single chromosome, then to a portion of the chromosome, and ultimately to a specific locus. Microsatellites are distributed unevenly in eukaryotic genomes, though, so the final stages of such a search often took months or years.

SNPs, which are distributed much more evenly and densely and can be tracked by the hundreds of thousands in a single experiment, offer obvious benefits for geneticists. "Our microsatellite business has been declining in parallel to how our SNP business has been growing," says Phoebe White, senior director of genotyping at Applied Biosystems (Foster City, California).

Even in traditional family-based linkage analyses, long the domain of microsatellite mapping, SNP profiling often provides a faster and cheaper path to gene discovery. Nonetheless, some geneticists prefer the traditional technique. "People are still doing linkage studies in families using microsatellite markers; microsatellites are very informative markers, they're just difficult to genotype," says Ann-Christine Syvanen, research group leader at the SNP Technology Platform (Uppsala University, Sweden).

Other researchers use microsatellites because they can't use SNPs. "Where we do see some microsatellite usage is in our [agricultural biotechnology] customers, where essentially the genomes may not be that well mapped, or there is no tried and true context sequence for the genome," says White. As more crop plants and other nonhuman organisms have their genomes characterized, she expects even these holdouts to migrate toward more SNP-based assays—and fewer microsatellite gels.

Syvanen, research group leader for the **SNP Technology Platform** at **Uppsala University** in Sweden.

Syvanen's facility, like several others scattered around the world, functions as a national-level service. Scientists in Sweden and other Scandinavian countries can send samples to the Uppsala center, which charges only its marginal operating costs to genotype up to 1 million SNPs per sample.

For the high-density analyses, the center uses the Illumina Beadstation with Illumina's GoldenGate and Infinium assays, while a GenomeLab SNPstream from **Beckman Coulter** setup handles projects with 12–384 SNPs per sample. "We have a facility that can analyze anything from a few SNPs or one SNP up to a million SNPs on chips, so we have the possibility to do any kind of SNP genotyping project really," says Tomas Axelsson, the Uppsala center's manager. Axelsson adds that the center is now doing everything from candidate gene screening and family-based linkage studies to large-scale GWA work for researchers all over the country.

Besides the expensive equipment, a SNP screening core also provides trained technicians and, critically, the bioinformatics infrastructure to handle floods of genotyping data. While the makers of SNP-screening chips generally build database software into their chip readers, most big facilities also run their own databases for more complex analyses.

Storing and studying genome-scale datasets also creates a serious hardware problem. A typical GWA study may fill 2 terabytes of hard disk space, and more recent studies that also incorporate whole-genome sequence information may take up to 15 terabytes per study. Even the declining cost of disk storage hasn't solved the problem, as the data must also be backed up and maintained in a permanent archive, a serious challenge for any type of computer data. "The technology for bioinformatics is evolving, but not the technology for storing the data," says Syvanen.

Chip makers are also aware of the informatics problems, and are starting to offer more sophisticated solutions. For example, in June, Illumina announced the launch of iControlDB, a database of controls for GWA studies. Regardless of the SNP profiling platform they're using, researchers can tap into this trove to see SNP profiles for thousands of controls that don't have a particular disease. The company hopes to have 20,000 control patients' SNP profiles in the database by the end of 2007.

Making a Federal Case of It

Basic researchers have been avid consumers of SNP screening technology, but most experts have also been expecting pharmaceutical companies to adopt the technology, as part of the push for pharmacogenomic therapies. Pharmacogenomics, which seeks to customize therapies based on patients' genotypes, could improve drug efficacy while simultaneously reducing the risk of side effects. Pharmaceutical companies are understandably circumspect about the details of their internal research programs, so until recently it was unclear how much SNP screening was really going on in industry.

"Over the past three years, we have gotten a number of voluntary genomic data submissions which were dealing with differential gene expression using microarrays. Over the past year or so, we have also started to get whole-genome association studies," says Federico Goodsaid, senior staff scientist in genomics at the **US Food and Drug Administration's** Center for Drug Evaluation and Research.

Under current FDA regulations, companies can submit genomic data voluntarily as part of a drug approval application, if they think the results will improve the agency's understanding of the product. Goodsaid reports seeing about "three to five" such submissions involving GWA so far. "But remember, we get to see something several months or maybe a year past the point at which people have started to do it," he adds, suggesting that a wave of GWA-based pharmacogenomic therapies may soon be headed for the clinic.

Indeed, equipment manufacturers are already anticipating a boom in clinical genotyping, with new systems that simplify the process and make it more robust. **Luminex,** in Austin, Texas, for example, now markets its adaptable xMAP bead-based flow cytometry system for a wide range of diagnostic genotyping applications, ranging from tissue typing to cardiac marker profiling. In the Luminex system, allele-specific oligonucleotides attached to microspheres serve as sensitive probes for targeted genotypes, which can be read quickly and reliably by flow cytometry.

Besides new gear, the next generation of clinical genotyping tests will also require clearer benchmarks. Because the standards for validating GWA-based biomarkers are still in flux, the FDA has now incorporated these studies into the second part of its Microarray Quality Control (MAQC) initiative. The MAQC's first phase focused on the reproducibility of gene expression experiments, but the second phase aims to standardize microarray data analysis. "The questions we had in MAQC I went back to how you ran the hybridizations and stuff like that, but in MAQC II, we are worried primarily with what happens after the data [are] there," says Goodsaid.

Currently, each GWA study develops and uses its own data analysis algorithms, so the MAQC II team plans to construct a matrix of tests, cross-applying different algorithms to different data sets and comparing the results. The project, which should be completed in 12 to 18 months, should help set the tone for a burgeoning field. "My guess is that we're going to be seeing a lot more [GWA] in the near future, because this is obviously a powerful technique for identifying different types of biomarkers," says Goodsaid.

Whether for developing pharmacogenomic therapies, searching for new disease-related genes in genomewide association studies, or performing traditional linkage analyses in families, SNPs are clearly revolutionizing genetics. For geneticists, the revolution has been a long time coming: after all, they've seen the whites of its eyes for nearly a century.

Alan Dove is a science writer and editor based in Massachusetts.

DOI:10.1126/science.opms.p0700018

Featured Participants

Affymetrix
www.affymetrix.com

Applied Biosystems
www.appliedbiosystems.com

Beckman Coulter
www.beckmancoulter.com

Illumina
www.illumina.com

Luminex
www.luminexcorp.com

Roche Diagnostics
www.roche-diagnostics.us

Uppsala University
www.uu.se/english

US Food and Drug Administration
www.fda.gov

New Products

Automated Multifunctional Image Analyzer

The Dyversity 4 multipurpose charge-coupled device (CCD) imaging system is suitable for laboratories that want to rapidly and accurately analyze images of one-dimensional and two-dimensional gels stained with commercial visible, fluorescent, or chemiluminescent dyes. Dyversity 4 comprises a high-resolution, 16-bit CCD camera inside a light-tight darkroom integrated to a computer containing Syngene's powerful image capture and analysis software. The darkroom comes complete with an overhead white light and features a programmable, moving stage that can be used with a range of gel sizes and can recall stage positions for each gel. It can be customized for specific applications by fitting filters and an ultraviolet transilluminator, blue and white light converters, and Cy dye lighting modules.

Syngene
For information +44(0)1223-727123
www.2dymension.com

Personal Genomics Website

With more than 2,000 human DNA variations and their medical consequences now cataloged and available, the SNPedia website is expanding to include analyses of personal genomic sequences, with the first of many being the sequences from J. Craig Venter and James Watson. Built on a wiki-platform that encourages community annotation and updating, SNPedia provides a valuable online resource to scientists, physicians, and students studying genotyping and genome variation, and even to lay individuals interested in knowing more about their own genomes.

SNPedia
For information 240-465-0788
www.snpedia.com

Deepwell Plates

Eppendorf Plate Deepwell 96 and 384 set new standards for purity, stability, and centrifuge and automation capability. With innovative, optimized well geometry, an exclusive error-safe labeling system, and "g-safe" stability up to 6,000 x g, the plates deliver first-rate results. The plates incorporate the new RecoverMax design with conical well bottoms and smooth, rounded corners and transitions for square well plates. These features provide faster pipetting, resuspension, and mixing because the liquid collects in the center of the well bottom, while wicking in well corners is prevented to eliminate cross-contamination between wells. Another innovation introduced in these plates is the OptiTrack matrix, which combines a choice of five vibrant border colors and a high-contrast, ultraviolet-resistant and chemical-resistant alphanumeric labeling system. This ensures simple plate orientation, even in stacks, and quick, error-safe identification of plates and wells.

Eppendorf
For information +44(0)1223-200440
www.eppendorf.com

Genotyping BeadChip

The Infinium Human-Linkage-12 Genotyping BeadChip takes advantage of the 12-sample format for linkage analysis. It offers low cost per sample for linkage analysis plus industry-leading call rates, uniform marker distribution, and superior single nucleotide polymorphism (SNP) content. This new addition to the Linkage portfolio complements the GoldenGate Human Linkage V Panel, which is suitable for degraded samples such as formalin-fixed, paraffin-embedded specimens. It is also available for researchers interested in conducting linkage analysis studies using Illumina's FastTrack Genotyping Service. Linkage analysis maps the location of disease-causing loci by identifying genetic markers that are co-inherited with the phenotype of interest. The 12-sample BeadChip and the flexibility of the Infinium Assay provide researchers with a cost-effective method for studying linkage with high information content.

Illumina
For information 858-332-4055
www.illumina.com

Cloning Ligation Kit

The Rapid DNA Dephos and Ligation Kit provides fast, convenient, and economical dephosphorylation and ligation of sticky-end or blunt-end DNA fragments, enabling ligation of DNA in 5 minutes and dephosphorylation in 10 minutes. The kit contains the new recombinant rAPid Alkaline Phosphatase, which is heat-inactivated in two minutes at 75°C, eliminating phenol-chloroform extraction steps and allowing dephosphorylation to be performed directly in restriction enzyme digests. Procedures are thus simplified for a broad array of applications, including cloning of fragments into plasmid or phage vectors, linker ligation, recircularization of linear vector DNA, and generation of libraries.

Roche Applied Science
For information +41-61-688-6983
www.roche-applied-science.com

Room Temperature Bacteria Storage

A new technology for storing and shipping *E. coli* at room temperature, CrudE is based on Biomatrica's proprietary synthetic chemistry technology, which preserves unpurified plasmid and genomic DNA harbored in *E. coli*. Bacterial cultures can be directly dried into CrudE, allowing for quick and efficient sample collection. Users simply add water to recover intact DNA ready for subsequent downstream applications. A sample management database tracks and organizes samples using barcode and radio-frequency identification tags that are integrated into CrudE individual tube, 96-well plate, and 384-well plate formats.

Biomatrica
For information 866-379-6879
www.biomatrica.com

POSITIONS OPEN

ASSISTANT/ASSOCIATE PROFESSOR - # 1405
Neurotoxicology
School of Pharmacy
University of Wyoming
College of Health Sciences - Academic Vacancy

The School of Pharmacy at the University of Wyoming invites applications for a nine-month tenure-track faculty position in the Division of Pharmaceutical Sciences at the level of **ASSISTANT/ASSOCIATE PROFESSOR of NEUROTOXICOLOGY.** We are seeking an individual with demonstrated expertise in areas of research that provide a fit to ongoing research in the Division of Pharmaceutical Sciences. Research in the area of neurotoxicology/neuropharmacology is expected, and particular focus may include: environmental neurotoxicity, neurotransmitter excitotoxicity, neurotoxicology associated with drugs, cellular/molecular approaches to study neuronal plasticity or neuroinflammation and developmental neurotoxicology. The position will also provide an opportunity to collaborate with the Center of Biomedical Research Excellence-Neuroscience Center, a multidisciplinary center with an outstanding Microscopy and Macromolecular Facility. The successful applicant is expected to develop and maintain an externally funded research program related to the applicant's area of expertise. The successful candidate will also be expected to teach courses in the toxicology/pharmacology curriculum, advise professional pharmacy and graduate students, participate in the Graduate Neuroscience Program, and provide service to the University, College, school, and the broader professional community.

Required qualifications include a Ph.D. (or equivalent) and demonstrated scholarly research and teaching experience. Postdoctoral experience is desirable but not required.

Salary and startup package will be competitive and commensurate with professional education, experience, and demonstrated abilities.

Interested candidates should submit a statement of research interests and objectives, a statement of teaching philosophy, curriculum vitae, representative publications and three letters of recommendation to: **Dr. M. Glaucia Teixeira, Toxicology Search Committee Chair, University of Wyoming, School of Pharmacy, Department 3375, 1000 E. University Avenue, Laramie, WY 82071.** Review of applications will begin November 15, 2007, and continue until a suitable candidate is identified.

The University of Wyoming is an Equal Opportunity/Affirmative Action Employer.

FACULTY POSITION for STEM CELL FACULTY RECRUITMENT
Department of Pharmacology University of Illinois at Chicago

The Department of Pharmacology at the University of Illinois College of Medicine (Chicago) is seeking candidates for an **ASSOCIATE PROFESSOR or PROFESSOR** appointment in the field of stem cell biology. Candidates should have a Ph.D. and/or M.D. degree, an outstanding publication record, and an NIH-funded research program in any on these areas: (1) stem cell biology, including cancer stem cells; (2) regenerative medicine; (3) molecular aspects of stem cell renewal/differentiation; (4) stem cell-based therapy. The Department has strong research and training programs **(website: http://www.uic.edu/depts/mcph/)** and consistently ranks among the top nationally. The successful candidate will have extensive opportunities for interdisciplinary collaboration, and a highly competitive startup package will be offered. Applications will be screened up to December 1, 2007. Position available July 2008. Please send by e-mail a single PDF file containing (1) curriculum vitae, (2) a summary of major research accomplishments and future research plan, (3) names, addresses, and e-mail addresses for three references to: **Attn: AP001 Search (e-mail: pharmjob@uic.edu), Department of Pharmacology (MC 868), University of Illinois at Chicago, College of Medicine, 835 S. Wolcott Avenue, Room E403, Chicago, IL 60612.** *UIC is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

FAU FLORIDA ATLANTIC UNIVERSITY

PH.D. PROGRAM in COMPLEX SYSTEMS and BRAIN SCIENCES PREDOCTORAL FELLOWSHIPS, RESEARCH and TEACHING ASSISTANTSHIPS

The aim of this Program is to train scientists who are both mathematically and biologically literate so that they can fully participate in multidisciplinary research to bring new ways of thinking into neuroscience. Individuals with undergraduate degrees in any pertinent discipline are invited to apply for this five-year training Program at the Florida Atlantics University (FAU) Center for Complex Systems and Brain Sciences.

Graduate training consists of a core curriculum in nonlinear dynamics, neuroscience, computational modeling, and cognitive science. Research areas include sensorimotor coordination and learning, human brain imaging, including functional magnetic resonance imaging, electroencephalogram, brainstem mechanisms of behavior, neural growth and development, cellular neurosciences, ion channel dynamics, speech production and perception, neurolinguistics, visual perception, music perception, and mathematics of complex systems.

Applicants should complete the application package that can be found on our website: http://www.ccs.fau.edu and send it together with a letter of interest, curriculum vitae, and three letters of reference to: **Rhona Frankel, Center for Complex Systems and Brain Sciences, BS-12, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431. E-mail: frankel@ccs.fau.edu.** Deadline: January 15, 2008. Additional mandatory FAU application can be found at website: http://graduate.fau.edu/GradApp/.

FACULTY POSITION
Behavioral Neuroscience

The Department of Psychology at the University of Memphis invites applications for a tenure-track faculty position at the **ASSISTANT PROFESSOR** level. Candidates must possess a Ph.D. and postdoctoral experience. While the specific area of specialization within behavioral neuroscience is open, preference will be given to candidates who have a strong record of research publications, currently have or are likely to obtain external funding, and have had experience in successful collaborative research. The person who fills the position will also be expected to teach both undergraduate and graduate students and to periodically teach a course in research methods or statistics. Our Department currently includes 32 full-time faculty and offers Ph.D. degrees in experimental, clinical, and school psychology. Evaluation of candidates will begin on November 15, 2007, and may continue until position is filled. Send curriculum vitae, three letters of recommendation, and reprints/preprints to: **Guy Mittleman, Chair, Faculty Search Committee, Department of Psychology, The University of Memphis, Memphis, TN 38152.** *The University of Memphis is an Equal Opportunity Affirmative Action Employer and encourages applications from women, ethnic minorities, and persons with disabilities.*

WILDLIFE DISEASE ECOLOGIST, WILDLIFE DEPARTMENT, Humboldt State University, California, tenure track, job #7411. Expertise in ecology of wildlife diseases, management of terrestrial vertebrates and Ph.D. required. Full-time teaching, graduate program, and other University/professional duties. Vacancy announcement at **website: http://www.humboldt.edu/~aps/employment/tenure.html.** *HSU is an Equal Opportunity/Title IX/ADA Employer.*

WPI

Department of Chemistry and Biochemistry Tenure Track Faculty Position

The Department of Chemistry and Biochemistry invites applications for a tenure-track position starting in August 2008. The candidate is expected to develop a vigorous, externally-funded research program at the interface of chemistry and biochemistry. This position is part of a new Life Science research initiative at WPI supported by a new state-of-the-art research facility. This $40M building hosts the Life Science research Departments and WPI Bioengineering Institute. The tenure-track position is at the ASSISTANT or ASSOCIATE level, depending on qualifications. The ideal applicant will have postdoctoral research experience, evidence of teaching excellence, an established record of research productivity, and demonstrated success or potential to secure research funding.

The Department offers undergraduate and graduate (Ph.D.) degrees in Chemistry and Biochemistry. WPI is a private, selective technological university with an undergraduate student body of 2800 and 1000 full-time and part-time graduate students. Worcester, New England's second largest city, offers ready access to diverse economic, cultural and recreational resources of the region. Further information about WPI and the department can be accessed at **http://www.wpi.edu.**

Applicants should send a curriculum vita, a statement of research and teaching interests, and the name of three references to: **Dr. José Argüello, Chair, Search Committee, Department of Chemistry and Biochemistry, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609.** Pdf formatted full applications can be sent to: **faculty-searchCBC@wpi.edu.** Review of applications will begin immediately and will continue until a suitable candidate is identified.

WPI offers a smoke free environment, competitive compensation and an excellent benefits package including health insurance, family tuition reimbursement and generous vacations.

To enrich education through diversity, WPI is an affirmative action, equal opportunity employer. Women and minorities are especially encouraged to apply.

Worcester Polytechnic Institute

A Member of the Colleges of Worcester Consortium

Beijing Institute of Genomics (BIG), Chinese Academy of Sciences

Full Professors, Associate Professors and 100 talents program professors

Director or Deputy-Director of Administrative Departments

Beijing Institute of Genomics (BIG) is one of the youngest institutions of Chinese Academy of Sciences, founded on the basis of Beijing Genomics Institute (BGI) and made significant contribution to the International Human Genome Project, International HapMap Project, International Chicken Genome Sequencing and Polymorphism Projects, as well as sequencing and analysis of the rice, silkworm and other important genomes.

At the moment of moving to the temporary campus close to the central site of the 2008 Olympic Games, BIG will open to the whole society its positions of 30 Full Professors/ Associate Professors, and of 8 Directors or Deputy-Directors of Administrative Departments. Willing to work in the planned section in Suzhou are encouraged. Details can be found at **http://www.big.ac.cn.**

Deadline of application: Review of the applications will begin November 8, 2007, and will continue until positions are filled. Contact: **gongkaizhaopin@big.ac.cn**

Huanming Yang, Ph.D.
Director-General, BIG, CAS

PENNSTATE University Park

Head, Department of Biochemistry and Molecular Biology

Penn State invites applications and nominations for the position of Head of the Department of Biochemistry and Molecular Biology. We are seeking an individual with a vibrant ongoing research program, strong interpersonal skills, a commitment to excellence, and a commitment to diversity and a supportive environment for faculty and students, to provide energetic and able leadership for the Department. The Department has 40 faculty members whose research explores a wide spectrum of biological questions at the molecular level. This research is supported by extensive external funding. The Department has vigorous undergraduate and graduate educational programs and participates in a number of interdisciplinary graduate programs. The Department interfaces with other excellent departments in the Eberly College of Science and has extensive collaborations with other life science researchers across Penn State, including at the Hershey Medical Center, as well as across the nation and around the world. Continued growth and excellence in life sciences is a priority of Penn State and the Eberly College of Science. The incoming head will have a number of faculty lines and other resources to enhance existing areas of expertise and develop new frontiers. Further information about the Department may be found at http://www.bmb.psu.edu.

The position is available for Fall 2008. Credentials appropriate to the rank of tenured full professor are required. Review of applications and nominations will begin November 15, 2007 and will continue until the position is filled. Applications, including curriculum vitae and the names of three references, and nominations may be submitted via email to mlb1@psu.edu or mailed to: BMB Head Search Committee, The Pennsylvania State University, Eberly College of Science, 512 Thomas Building, University Park, PA 16802.

Penn State is committed to affirmative action, equal opportunity and the diversity of its workforce.

PENN STATE ***Making Life Better***

HARVARD UNIVERSITY

DEPARTMENT OF CHEMISTRY AND CHEMICAL BIOLOGY

ASSISTANT PROFESSORSHIP IN CHEMISTRY

Applicants are invited to apply for tenure-track assistant professorships in all fields of chemistry. Applicants should arrange to have three letters of recommendation sent independently and should provide a curriculum vitae, a list of publications and an outline of their future research plans.

All applications and supporting materials must be submitted via:
http://www.lsdiv.harvard.edu/ccb/facultysearch/

The deadline date for receipt of applications and supporting materials is **November 1, 2007**.

Harvard University is an Affirmative Action, Equal Opportunity Employer. Applications from and nominations of women and minority candidates are strongly encouraged.

NATIONAL CANCER INSTITUTE

Laboratory of Pathology
Molecular Diagnostics Laboratory
Staff Scientist

The Laboratory of Pathology, National Cancer Institute, Bethesda, MD is seeking to hire a dynamic scientist/technologist to serve as the technical supervisor for our Molecular Diagnostics Laboratory. The laboratory provides specialized gene-based diagnostic testing focused in oncology, and is currently staffed by two highly skilled molecular technologists. In addition, the laboratory works closely with NCI investigators and clinicians to develop novel gene-based molecular tests tailored to the requirements of ongoing clinical protocols.

The successful candidate will be responsible for all aspects of the daily work of the laboratory. Duties include provision of both technical and administrative support to the laboratory and ensuring high quality diagnostic services. Specific responsibilities include, performance of tests, assignment, oversight, and review of the work of each medical technologists, and ensuring compliance with regulatory standards (CAP and CLIA). The incumbent will also be responsible for monitoring the laboratory budget, building a positive work climate, and maintaining records. In addition the incumbent will be expected to participate in the education of fellows and residents, pre and postdoctoral students as well as the medical and scientific staff.

The successful candidate must demonstrate a strong record in clinical, translational or basic research and will be expected to pursue active research and participate in the developmental research program of the laboratory. The incumbent should be able to independently develop and implement new procedures and tests that will be beneficial to the laboratory, the patient population served, NCI clinical protocols, and the academic mission of the laboratory and the NCI.

The successful candidate should have a doctoral degree and broad experience in molecular biologic technologies. Candidates without a doctoral degree, but with an outstanding resume may be considered. Experience with nucleic acid extraction, analytic methods including PCR (regular, RT, quantitative) and blotting techniques, DNA sequencing, and other unique chemistry platforms is required, as is experience with database and spreadsheet based software. In addition, it is highly desirable that the applicant be familiar and facile with automated equipment such as sequencers and real-time PCR analyzers. Experience in the development and maintenance of laboratory records, cost analysis, and strategies for methods development and validation is also desirable.

Applications will be accepted until **November 21, 2007**. Interested individuals should contact: **Mark Raffeld, M.D., Chief, Specialized Diagnostics, Building 10, Room 2N110, Laboratory of Pathology, NCI, 9000 Rockville Pike, Bethesda, MD 20892, Fax: 301-402-2415, Phone: 301-496-1569**.

NATIONAL CANCER INSTITUTE

Tenure Track Investigator

The Lab of Receptor Biology and Gene Expression, Center for Cancer Research, National Cancer Institute, is recruiting for a position in the area of chromatin structure and function, chromosome biology, and nuclear architecture. The position is at the level of Tenure Track Principal Investigator, but senior investigators with expertise in the program area may be considered. Applicants should have a Ph.D. or M.D. degree, a strong publication record, and demonstrated potential in creative research. Salary will be commensurate with education and experience. Applications should be submitted as electronic files (Word or pdf docs), and should include a curriculum vitae, statement of research interests, and 3 letters of recommendation. Submit by **Nov. 30, 2007** to: **Christine Koch-Paiz, Lab of Receptor Biology & Gene Expression, National Cancer Institute, NIH, Bethesda MD 20892-5044, paizc@mail.nih.gov**.

NATIONAL CANCER INSTITUTE

TENURE TRACK POSITION IN IMMUNOLOGY

The Experimental Immunology Branch (EIB), Center for Cancer Research (CCR) of the National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS), Bethesda, MD, invites applicants to apply for a tenure track position in Immunology. The applicant should have a Ph.D., M.D. or equivalent degree, a strong record of scientific accomplishments, and the potential to establish an independent research program in any aspect of molecular or cellular immunology. The position provides salary and full funding to establish an independent research program, including laboratory space, equipment, budget, technical personnel, and support for fellows. Salary will be commensurate with education and experience. The EIB consists of 9 Principal Investigators: Alfred Singer, Triantafyllos Chavakis, Richard Hodes, Andre Nussenzweig, Paul Roche, David Segal, Stephen Shaw, Gene Shearer and Dinah Singer. Active research covers a wide range of areas of immunology including: thymic education and T cell differentiation, HIV-induced immunodeficiency, genetic recombination and chromosomal instability, inflammation biology, antigen presentation, receptor assembly and transport, signal transduction, and regulation of gene expression. Scientific interactions are encouraged and occur extensively among members of EIB as well as with other scientists at the NIH. Applicants should send a CV and bibliography, outline of a proposed research program (no more than two pages), and three letters of recommendation to **Caroline McCabe, 10 Center Drive, Bldg. 10, Room 4B36, NIH, Bethesda, MD 20892-1360**. Applications must be received by **December 15, 2007**.

Positions @ NIH

THE NATIONAL INSTITUTES OF HEALTH

Investigator Recruitment in Cancer Genetics

National Human Genome Research Institute

The Cancer Genetics Branch (CGB) of the National Human Genome Research Institute (NHGRI) is seeking to recruit an outstanding tenure-track investigator to pursue innovative, independent research in cancer genetics. General areas of interest include, but are not limited to:

- Cancer Gene Discovery
- Comparative Cancer Genomics
- Genetic Epidemiology
- Molecular Profiling of Tumors
- Functional Genomics of Cancer
- Genome Instability in Cancer
- Markers for Early Detection
- Genetics of Tumor Progression

The successful candidate will be able to take advantage of interactions with a highly collegial group of scientists within NHGRI and the NIH campus as a whole. In addition, they will have access to NHGRI's outstanding core laboratories, as well as the unparalleled resources of the NIH Clinical Center.

Candidates must have a Ph.D., M.D., or equivalent degree, as well as comprehensive, advanced training and a record of accomplishment in one of the targeted areas. This position includes generous start-up funds, an ongoing commitment of research space, laboratory resources, and positions for personnel and trainees.

Interested applicants should submit a *curriculum vitae*, a three-page description of their proposed research, and three letters of recommendation through our online application system at http://research.nhgri.nih.gov/apply. The closing date for applications is November 16, 2007.

For more information on CGB and NHGRI's Intramural Program, please see http://www.genome.gov/DIR. Specific questions regarding the recruitment may be directed to Dr. Larry Brody, the Search Chair, at lbrody@helix.nih.gov. Questions may also be directed to Dr. Elaine Ostrander, the CGB Branch Chief, at eostrand@mail.nih.gov.

Interested applicants should also be aware of two concurrent tenure-track faculty searches being conducted by NHGRI's Genetics and Molecular Biology Branch (GMBB) and Genetic Disease Research Branch (GDRB). Information on these searches may be found at http://genome.gov/11509039. Qualified candidates are welcome to apply for multiple searches; please note that a separate application must be filed for each search for which you wish to be considered.

DHHS and NIH are Equal Opportunity Employers and encourage applications from women and minorities.

NATIONAL HUMAN GENOME INSTITUTE Divison of Intramural Research

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR

Investigator Recruitment in Genetics and Molecular Biology

National Human Genome Research Institute

The Genetics and Molecular Biology Branch (GMBB) of the National Human Genome Research Institute (NHGRI) is seeking to recruit outstanding tenure-track investigators to pursue innovative, independent research. The faculty within GMBB are highly interactive and use a variety of approaches to understand and develop new treatments for genetic and complex diseases. Current areas of research include hematopoiesis, the genetic disorders of red blood cells, cellular immunology, gene therapy, leukemogenesis, genomic instability in disease, the genetics of skin diseases, and the genomics of the skin microbiome. Of particular interest are candidates with research interests in the following areas:

- Clinical genetics
- Application of genomics tools to understanding human disease
- Clinical/translational research
- Basic genetic/genomics research

The successful candidate will be able to take advantage of interactions with a highly collegial group of scientists within NHGRI and the NIH campus as a whole. In addition, the successful candidate will have access to NHGRI's outstanding core laboratories, as well as the unparalleled resources of the NIH Clinical Center.

Candidates must have a Ph.D., M.D., or equivalent degree, as well as comprehensive, advanced training and a record of accomplishment in one of the targeted areas. This position includes a generous start-up allowance, an ongoing commitment of research space, laboratory resources, and positions for personnel and trainees.

Interested applicants should submit a *curriculum vitae*, a three-page description of proposed research, and three letters of recommendation through our online application system, at http://research.nhgri.nih.gov/apply. The closing date for applications is November 16, 2007.

For more information on GMBB and NHGRI's Intramural Program, please see http://genome.gov/DIR. Specific questions regarding the recruitment may be directed to Dr. Fabio Candotti, the Search Chair, at fabio@nhgri.nih.gov. Questions may also be directed to Dr. David Bodine, the GMBB Branch Chief, at tedyaz@nhgri.nih.gov. Interested applicants should also be aware of two concurrent tenure-track faculty searches being conducted by NHGRI's Cancer Genetics Branch (CGB) and Genetic Disease Research Branch (GDRB). Information on these searches may be found at http://genome.gov/11509039. Qualified candidates are welcome to apply for multiple searches; please note that a separate application must be filed for each search for which you wish to be considered.

DHHS and NIH are Equal Opportunity Employers and encourage applications from women and minorities.

NATIONAL HUMAN GENOME INSTITUTE Divison of Intramural Research

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR

THE NIH IS DEDICATED TO BUILDING A DIVERSE COMMUNITY IN ITS TRAINING AND EMPLOYMENT PROGRAMS

WWW.NIH.GOV

Investigator Recruitment in Genetic Disease Research

National Human Genome Research Institute

The Genetic Disease Research Branch (GDRB) of the National Human Genome Research Institute (NHGRI) is seeking to recruit outstanding tenure-track or tenured investigators to pursue innovative, independent research. GDRB faculty members are highly interactive and use a variety of approaches to study the genes and proteins involved in normal development and related disease processes. Investigators in the Branch are currently engaged in studies of limb and skeletal development, pigment cell development and neurocristopathies, T-cell development, function, and defects in host defense; and the role of rare variants in common disease. Of particular interest are candidates with backgrounds in one or more of the following areas:

- Clinical or translational research
- Molecular and genomic approaches to understanding the mechanisms of disease
- Basic genetic or genomic research

The successful candidate will be able to take advantage of interactions with a highly collegial group of scientists within NHGRI and the NIH campus as a whole. In addition, the successful candidate will have access to NHGRI's outstanding core laboratories, as well as the unparalleled resources of the NIH Clinical Center. Candidates must have a Ph.D., M.D., or equivalent degree, as well as comprehensive, advanced training and a record of accomplishment in one of the targeted areas. This position includes a generous start-up allowance, an ongoing commitment of research space, laboratory resources, and positions for personnel and trainees. Interested applicants should submit a *curriculum vitae*, a three-page description of proposed research, and three letters of recommendation through our online application system, at http://research.nhgri.nih.gov/apply. The closing date for applications is November 16, 2007.

For more information on GDRB and NHGRI's Intramural Program, please see http://genome.gov/DIR. Specific questions regarding the recruitment may be directed to Dr. William Pavan, the Search Chair, at bpavan@nhgri.nih.gov. Questions may also be directed to Dr. Leslie Biesecker, the GDRB Branch Chief, at leslieb@nhgri.nih.gov. Interested applicants should also be aware of two concurrent tenure-track faculty searches being conducted by NHGRI's Cancer Genetics Branch (CGB) and Genetics and Molecular Biology Branch (GMBB). Information on these searches may be found at http://genome.gov/11509039. Qualified candidates are welcome to apply for multiple searches; please note that a separate application must be filed for each search for which you wish to be considered.

DHHS and NIH are Equal Opportunity Employers and encourage applications from women and minorities.

NATIONAL HUMAN GENOME INSTITUTE Divison of Intramural Research

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES | NATIONAL INSTITUTES OF HEALTH | genome.gov/DIR

Clinical Tenure-Track Position
in Malaria Pathogenesis and Human Immunity
Laboratory of Malaria and Vector Research

The National Institute of Allergy & Infectious Diseases (NIAID), Division of Intramural Research (DIR), Laboratory of Malaria & Vector Research (LMVR), located in Rockville, MD is seeking an outstanding tenure-track investigator to develop a clinical research program in malaria pathogenesis and human immunity that includes field and laboratory studies. A tenure-track investigator is equivalent to an Assistant or Associate Professor in a University setting.

The LMVR conducts high-impact, innovative scientific research on malaria and the mosquitoes responsible for its transmission. Overseas field work in Africa and Southeast Asia is integral to these activities. Results from LMVR research lead to new information on parasite and host factors that affect malaria transmission and infection, biological responses that affect the course of disease, and genetic determinants that affect outcomes in vaccine trials and drug treatment.

The successful candidate will implement and direct a vigorous, independent research program in malaria pathogenesis and immunity with both laboratory and overseas clinical components. The incumbent will develop clinical protocols that may include, but are not limited to, research on the natural history and pathophysiology of malaria, genetic determinants of disease virulence and outcome, acquired and innate antimalarial immunity, or research on factors that affect the outcome of malaria interventions. An outstanding postdoctoral record of research accomplishment and M.D., or equivalent degree is required for this position; board eligibility/board certification in infectious diseases is highly desirable. The incumbent will be expected to maintain a current license to practice medicine and to hold credentialing with the NIH Clinical Center.

Independent resources including space, support personnel, and an allocated annual budget for services, supplies and salaries have been committed to the position. Facilities at existing NIAID field sites in Africa and Asia will be available to the incumbent, and support for field collaborations at new sites is possible. This is a tenure-track appointment under Title 42. Salary is dependent on experience and qualifications.

Interested candidates may contact **Dr. Thomas Wellems, Chief, LMVR via email at twellems@niaid.nih.gov** for additional information about the position.

To apply for the position, send your curriculum vitae, bibliography, an outline of your proposed research program (no more than two pages), and list of 3-5 most significant research papers by **December 3, 2007** via email to **Ms. Wanda Jackson at jacksonwa@niaid.nih.gov**. In addition, three letters of recommendation must be sent to **Chair, LMVR Search Committee c/o Ms. Wanda Jackson, 10 Center Drive MSC 1356, Building 10, Room 4A-26, Bethesda, Maryland 20892-1356**. E-mail is preferred. Please note search **#017** when sending materials. Further information on working at NIAID is available on our website at: **http://healthresearch.niaid.nih.gov**.

UMKC

UNIVERSITY OF MISSOURI-KANSAS CITY
SCHOOL OF BIOLOGICAL SCIENCES

Professor and Head, Division of Cell Biology and Biophysics

Applications are invited for the Head of the Division of Cell Biology and Biophysics at the School of Biological Sciences, University of Missouri-Kansas City. The successful candidate should have a proven record of sustained externally funded research, scholarly activity, and leadership potential. The candidate will be expected to participate in graduate and/or undergraduate teaching, faculty mentorship, and work closely with the Dean on decision-making matters pertaining to the growth, development and direction of the School. The School of Biological Sciences is positioning itself to become a regional leader in the areas of structural biology and molecular cell biology and welcomes applications from qualified candidates in these research areas; however, outstanding scientists from all areas of basic life sciences research are encouraged to apply. The successful candidate will receive a competitive 12-month salary, renovated research space, a start-up package commensurate with rank, and the availability of excellent research support facilities within the School of Biological Sciences. Candidates should have a Ph.D. degree and currently be in a tenured academic position at the rank of Professor.

Please direct all inquiries or nominations to **Dr. Lawrence A. Dreyfus**, Dean, School of Biological Sciences (**dreyfusl@umkc.edu**). To apply, please submit electronically (MS Word or pdf) a CV, a statement of present and future research interests, and the names and addresses of 3 references to: **dreyfusl@umkc.edu**. All materials will be handled with strict confidentiality. The position will remain open until filled.

UMKC is an Affirmative Action/Equal Opportunity Employer.
Women, minorities, veterans, and individuals with disabilities are encouraged to apply.

FACULTY POSITION IN BIOCHEMISTRY

Kansas City University of Medicine and Biosciences invites applications from outstanding candidates for appointment in the Department of Biochemistry at the rank of **Assistant** or **Associate Professor**. As we continue to expand our research capacity, we seek candidates with research interests in the chronic diseases of aging, particularly diseases associated with protein misfolding; applicants who can produce new knowledge in the areas of chemical neurobiology using innovative techniques are especially encouraged to apply. The successful applicant will have a Ph.D. (or equivalent doctorate), a record of scholarly publications and progressive external funding, be willing to mentor graduate and medical students and to contribute to a novel instructional curriculum. For additional information, contact **Norbert W. Seidler, Ph.D., Chair, Department of Biochemistry, 1-800-234-4847, ext. 2207 or 816-283-2207, nseidler@kcumb.edu**. Please visit **www.kcumb.edu** and click on 'Employment' to view remainder of ad and for CV submission directions.

KANSAS CITY UNIVERSITY
MEDICINE & BIOSCIENCES

Philadelphia College of Osteopathic Medicine (PCOM) brings to light a rich tradition of excellence in education and leadership. Work in an atmosphere and culture that is open and receptive to fresh ideas and new perspectives. Here, sparked by ideas and innovations that continue to shape the future of osteopathy, a myriad of professionals turn Ambition into Accomplishment.

Faculty Positions

Assistant Professor
Georgia Campus

We are seeking a full time Assistant Professor (Associate will be considered) for our Division of Basic Sciences dept. at our Suwanee, GA campus. Candidates will engage in scholarly activity by pursuing research activities that will support graduate program development, including mentoring students, publication of works and securing extramural funding to support independent research program. Candidates must have PhD in respective field or closely related area. 3 years Postdoctoral experience preferred.

Anatomy/Cellular Biology
Georgia Campus

Candidates for this full time faculty position also in Suwanee, GA, should be trained in an area of human anatomy/cellular and molecular biology and capable of teaching Gross Anatomy (including dissection) as well as participate in the teaching of medical cell biology in the medical curriculum and the Graduate Program in Biomedical Sciences.

PCOM's beautiful Suwanee campus is Georgia's newest medical college with a total enrollment of 255 students in its first year DO classes and 66 biomedical sciences graduate students. The campus is located just 38 miles from the airport and 33 miles downtown to Atlanta.

Please send letter of interest and curriculum vitae to: **Philadelphia College of Osteopathic Medicine, Human Resources Department, 4190 City Avenue, Philadelphia, PA 19131, Email: HR@pcom.edu, Fax: 215-871-6505.** EEO

PHILADELPHIA COLLEGE OF OSTEOPATHIC MEDICINE
WWW.PCOM.EDU

Duke University seeks applications for open rank, tenure track positions in the broad field of **cellular systems biology**. We seek applicants from both **experimental** and **quantitative/computational** disciplines with research interests in the molecular bases of cellular function, development, and evolution. These new appointments will substantially enhance existing Duke strengths in experimental and modeling approaches to understanding the complexity of genetic, metabolic, and signaling networks. Successful applicants will have appointments in one or more Duke departments based on mutual interests. All appointees will be affiliated with the **Duke Center for Systems Biology**, a cross-school, campus-wide academic center that is also one of the NIH-supported National Systems Biology Centers.

Applicants should submit a curriculum vitae, a brief summary of current and proposed research, reprints of 2 or 3 key publications and a statement of teaching interests via the web at **www.academicjobsonline.org**. Junior candidates should arrange for three letters of recommendation to be uploaded to this website or sent directly to: **Systems Biology Search, Duke University, Box 90338, Durham, NC 27708-0338**. Senior candidates should give the names of three potential referees. Application review will begin on **1 December 2007**, and continue until the positions are filled.

Duke University is an Equal Opportunity/ Affirmative Action Employer; women and members of minority groups are strongly encouraged to apply.

McMaster University
HEALTH SCIENCES

McMaster University announces
Canada's Premier Postdoctoral Fellowships
A new opportunity to pursue top-level research training

Applications are now being accepted for the Michael G. DeGroote Fellowships, prestigious new funding opportunities for post-doctoral candidates in health sciences research. The awards are offered through the Faculty of Health Sciences at McMaster University. There are two types of awards being offered:

Michael G. DeGroote Post-Doctoral Research Award
This award, one of the largest of its kind in the world, will provide advanced training for outstanding individuals who have completed a post-doctoral fellowship and are seeking a junior faculty position at McMaster University. Through the generosity of Michael G. DeGroote, the award will provide $75,000 Cdn per annum for two years for a Research Associate in the Faculty of Health Sciences at McMaster University. Extensive training will be provided to the successful candidate to develop a competitive research program. Upon completion of the fellowship, the recipient may be recommended for an Assistant Professor position at McMaster.

Michael G. DeGroote Post-Doctoral Fellowship Award
These prestigious awards are designed for candidates who have an exemplary academic record and are interested in pursuing post-doctoral work in one of the numerous areas of research strengths in the Faculty of Health Sciences at McMaster University. Also funded through the generosity of Michael G. DeGroote, the award will provide annual support of up to $45,000 per annum for up to three years.

Successful candidates for these awards will have the opportunity to work with Health Sciences faculty who are conducting leading-edge research and are considered among the best in their fields. They will have access to state-of-the-art facilities that enable progressive research designed to impact health care throughout the world.

Application deadline for both awards is **January 1, 2008**.

McMaster University is consistently rated as the most research-intensive university in the country. Its affiliation with Hamilton's teaching hospitals and research institutes provides an exceptional environment for interdisciplinary research within a fully integrated academic health network.

More information, including frequently asked questions, information on the city of Hamilton and on McMaster University and its research strengths, can be found at http://fhs.mcmaster.ca/mgdfa or www.fhs.mcmaster.ca

Michael G. DeGroote SCHOOL OF MEDICINE McMaster University | School of Rehabilitation Sciences | School of Nursing *Lead. Engage. Inspire.*

For more information about the Faculty, visit our web-site at **www.fhs.mcmaster.ca**

Dr. Peter George
President and Vice-Chancellor, McMaster University

Dr. John Kelton
Dean and Vice-President, Faculty of Health Sciences

An equal opportunity employer
McMaster University is committed to Employment Equity and encourages applications from all qualified candidates, including women, aboriginal people, people with disabilities, sexual minorities, and visible minorities. In accordance with Canadian immigration requirements, Canadian citizens and permanent residents of Canada will be considered first.

TOGETHER, ADVANCING HEALTH THROUGH LEARNING AND DISCOVERY

HARVARD MEDICAL SCHOOL

Professor and Chair

DEPARTMENT OF NEUROBIOLOGY
NATHAN MARSH PUSEY PROFESSOR OF NEUROBIOLOGY

Harvard Medical School is seeking an exceptional academic and scientific leader to serve as the Nathan Marsh Pusey Professor of Neurobiology and Chair of the Department of Neurobiology. The successful candidate will be an internationally recognized neuroscientist and visionary leader who thinks broadly about the full range of basic and translational neuroscience research. The individual should be an exemplary teacher, dedicated to medical and graduate education and to the mentoring of junior faculty. The candidate should be highly motivated to promote broad collaboration across Harvard Medical School, including its affiliated institutions as well as the larger University community. Highly effective administrative skills and experience in the management of complex research and/or educational programs is highly desirable. We seek an outstanding academic neuroscientist who is poised to lead one of the nation's premiere departments into the future.

Interested individuals should send a letter of application and current CV to:

Jeffrey S. Flier, MD
Dean, Harvard Medical School
c/o Office for Faculty Affairs
25 Shattuck Street
Gordon Hall-206
Boston, MA 02115
E-mail: neurobiology_search@hms.harvard.edu

Harvard Medical School is an equal opportunity/affirmative action employer with a strong institutional commitment to diversity in their faculty. Women and minority candidates are particularly encouraged to apply.

DEPARTMENT CHAIR

Chemistry

The University of Alabama seeks an outstanding individual for the position of Chair of the Department of Chemistry. The UA Department of Chemistry is housed in the new Shelby Hall and comprises 22 faculty members in the five traditional areas of specialization with research programs and center activities focused on synthesis, material science, biological systems, and energy. The UA Chemistry program places emphasis on quality education at the undergraduate and graduate levels while building a strong research enterprise with private and public sector collaborators. The successful candidate must possess exceptional leadership abilities, have demonstrated administrative experience, and have an understanding and enthusiasm for both the teaching and research missions. The area of expertise of the applicant is open, but should complement those of the existing faculty and future plans for growth in the Department. Further information about the Department and its Faculty is available on our website at **http://bama.ua.edu/~Chem**

All candidates should provide a curriculum vita (including publication list), the names of at least 3 references, as well as statements of administrative and leadership philosophy, research plans, and teaching philosophy and interests. Applications (hardcopy only) and nominations should be sent to: Dr. Carolyn J. Cassady, Chairperson Chemistry Chair Search Committee; Department of Chemistry; The University of Alabama; Box 870336; Tuscaloosa, AL 35487-0336. Potential candidates may contact the chairperson of the search committee at ccassady@bama.ua.edu if additional information is desired. Consideration of applications begins on November 30, 2007 and continues until the position is filled.

The University of Alabama is an Affirmative Action/Equal Opportunity Employer. Applications from women and minorities are encouraged.

Crimson is
THE UNIVERSITY OF ALABAMA

MICHIGAN STATE UNIVERSITY

Two Faculty Positions in Human Genetics

Two faculty positions in human genetics are available at Michigan State University (MSU) as part of a campus-wide initiative to increase human genetics research on campus. Individuals are sought for one junior level (assistant professor) and one senior level position.

Research will address fundamental questions of human genetics. At least one position is intended for a candidate having research experience in statistical genetics/genetic epidemiology with an emphasis on complex diseases. In addition, a laboratory-based human geneticist is sought.

MSU offers a highly collegial, interdisciplinary environment with many collaborative opportunities through two MSU community-based medical schools (MD and DO) associated with large health care systems in six cities, four genetics diagnostic labs (DNA, cytogenetics, prenatal screening, HLA), a genetic counseling center, strong epidemiology, cell & molecular biology and neuroscience programs, an interdepartmental graduate program in genetics, and strength in research in a wide array of basic sciences. Michigan State provides excellent research core facilities in genomics, proteomics, and microscopy.

Each successful candidate may choose to be affiliated academically with one or more departments, including Microbiology & Molecular Genetics, Pediatrics & Human Development, Epidemiology, Biochemistry & Molecular Biology, Pathobiology & Diagnostic Investigation, Physiology, Psychology, Pharmacology & Toxicology and Statistics and Probability.

These are academic-year, tenure-track, research-oriented positions, with moderate teaching responsibility. A doctoral degree (PhD, MD, DVM, or DO) and research experience are required. Salary will be commensurate with experience. Applicants should submit a letter of application, curriculum vitae, statement of research goals, copies of pertinent reprints and contact information (address, e-mail, and phone) for three referees to the following address: **Human Genetics Search Committee, Genetics Program, 2240 Biomedical & Physical Sciences Building, Michigan State University, East Lansing, MI 48824.** Digital submissions in pdf format to **genetics@msu.edu** are encouraged. Review of applications will begin in November, 2007 and will continue until the position is filled.

Michigan State University is committed to achieving excellence through cultural diversity. The university actively encourages applications and/or nominations from women, persons of color, veterans and persons with disabilities.

MSU IS AN AFFIRMATIVE ACTION, EQUAL OPPORTUNITY EMPLOYER.

CLINICAL ASSISTANT PROFESSOR OF ENVIRONMENTAL BIOLOGY

Department of Biology

ARTS AND SCIENCE

The Department of Biology at New York University invites applications for a clinical assistant professor appointment to start September 1, 2008, pending budgetary and administrative approval. Responsibilities include developing and teaching in the Department's undergraduate curriculum in Environmental Biology, a new minor available from the Department and a complementary component of the University's new Environmental Studies Program. Teaching duties will include six courses annually. Previous research and teaching experience is preferred. The Department of Biology (http://www.nyu.edu/fas/dept/biology) offers an outstanding and collegial research environment. Opportunities exist for active collaborations with related divisions within the University and other institutions in the New York metropolitan area, including the New York Botanical Garden and the American Museum of Natural History.

Candidates should submit applications, including a CV and three letters of reference. *Review of applications will begin immediately with a final deadline of* ***December 31, 2007***. Electronic applications as PDF files should be submitted to **biology.recruitment@nyu.edu** using the following address in the cover letter, **Chair of the Environmental Biology Search Committee, Department of Biology, New York University, 1009 Silver Center, 100 Washington Square East, New York, NY 10003. Closing date: December 31, 2007.**

NEW YORK UNIVERSITY

NYU is an Equal Opportunity/Affirmative Action Employer.

RESEARCH OPPORTUNITIES

VIRGINIA BIOINFORMATICS INSTITUTE

Assistant, Associate and Full Professorships at the Virginia Bioinformatics Institute: Laboratory-Centered and Computationally-Centered

The Virginia Bioinformatics Institute (VBI) at Virginia Tech has faculty openings for assistant, associate and full professorship levels. VBI is a world-class research institute in the life sciences, integrating theory, modeling, simulation and wet laboratories in a transdisciplinary, team research model. Areas of strength among the 18 research groups at VBI include infectious diseases, ranging from the molecular to the population scale, systems biology approaches to study stress response in several organisms, modeling and simulation of biological and other networks, functional genomics, metabolomics, proteomics and bioinformatics/computational/synthetic biology. To support growth of transdisciplinary research, we especially seek scientists with significant wet lab operations, quantitative social scientists or human-computer interaction faculty. Successful candidates at all levels are expected to have an established research program and a strong track record of substantial extramural research funding.

About VBI. Established in 2000 by the Commonwealth of Virginia, the Institute is a part of Virginia Tech (VT) and has its own 130,000 sq ft research facility with state-of-the-art core laboratory and computational facilities as well as new facilities in the Washington, D.C. area (in Alexandria, VA). VBI strongly emphasizes team science and organizes research outside of boundaries of academic disciplines. Research programs represented at VBI assemble to meet the specific needs of those programs; it is a flexible environment that rewards the notion of a problem-solving capability on the move. Extensive national and international collaborations complement the expertise of the faculty, including strong interactions with several biomedical research centers. Faculty entrepreneurial activities are strongly encouraged and the Institute and the university provide support for the establishment of commercial ventures.

VBI's facility in Alexandria is an integral part of Virginia Tech's expansion into that region. Faculty members whose programs will not require proximity to laboratory facilities will have the option of basing their primary research efforts there while still accessing VBI's state-of-the-art wet laboratory facilities in Blacksburg. Exceptional new faculty may also have the option of joint affiliations with other departments at Virginia Tech and two prominent medical schools on the East Coast. Reference posting 061384. For more information on faculty positions, please contact the Head of the Faculty Search Committee João Setubal (Tel: 540 231 9464; setubal@vbi.vt.edu)

Along with a strong research environment, the Institute actively participates in "Genetics, Bioinformatics, and Computational Biology" (GBCB), an interdepartmental Ph.D. program that emphasizes both computational and experimental sciences, and which attracts outstanding students from diverse disciplinary backgrounds.

Other Research Opportunities at VBI:

Research Groups

- Bioinformatics Scientist – Computational Biologist, posting 070821 (Sobral)
- Molecular Biologist and Laboratory Manager, posting 070666 (Peccoud)
- Postdoctoral Associate: Aptamer Discovery, posting 070668 (Peccoud)
- Postdoctoral Associate: HIV Replication, posting 070354 (Peccoud)
- Postdoctoral Associate: Microfluidics and Mass Spectrometry Specialist, posting 070670 (Lazar)
- Program Manager or Senior Program Manager, posting 070996 (Peccoud)
- Senior Simulation Science Software Developer, posting 070225 (Barrett)

For information on research in VBI faculty groups please consult www.vbi.vt.edu/public_relations/annual_reports/2007_scientific_annual_report

Core Facilities

- IT Project Manager, posting 070659 (Core Computational Facility)
- Portal Development Specialist (multiple openings), posting 070656 (Core Computational Facility)
- Proteomics Specialist, posting 070724 (Core Laboratory Facility)
- Systems Administrator, posting 070769 (Core Computational Facility)

For more information on VBI core facilities please consult www.vbi.vt.edu

For more Information:

- To apply, visit www.jobs.vt.edu and search by posting number
- To learn more about the Interdisciplinary PhD program in Genetics, Bioinformatics, and Computational Biology (GBCB), visit http://www.grads.vt.edu/academics/programs/gbcb/
- To learn more about VBI, please visit www.vbi.vt.edu

VBI

VirginiaTech
Invent the Future

ASSISTANT PROFESSOR

Integrative Animal Physiology

The Department of Biological Sciences at the University of Alabama invites applications for a tenure-track position at the rank of assistant professor in integrative animal physiology to begin August 2008. Applicants must have a Ph.D. and post-doctoral research experience. Candidates who study signaling mechanisms that underlie physiological responses in either invertebrate or vertebrate systems are particularly encouraged to apply. The successful candidate will be expected to establish an active, externally-funded research program that includes undergraduate and graduate student mentoring. The successful applicant will contribute to the teaching mission of the department in the areas of physiology, anatomy, and their area of specialization such as endocrinology.

To apply, mail hardcopies of *curriculum vitae*, a letter of application that includes your research interests and goals, a statement of teaching philosophy, a list of courses in your area of expertise, and have three letters of reference sent to: Search Committee - Integrative Animal Physiology, Department of Biological Sciences, Box 870344, The University of Alabama, Tuscaloosa, AL 35487. Questions about the position may be addressed to Dr. Stephen Secor, Chair of the Search Committee at **ssecor@biology.as.ua.edu** or 205-348-1809. Review of applications will begin December 3, 2007, and continue until the position is filled.

For more information visit our website at
http://www.as.ua.edu/biology

The University of Alabama is an Affirmative Action/Equal Opportunity Employer. Applications from women and minorities are encouraged.

Crimson is
THE UNIVERSITY OF ALABAMA

The **Department of Immunobiology** of the **Yale University School of Medicine** seeks applications for a senior faculty (**Associate** or **Full Professor**) position to develop a new research program within the Human and Translational Immunology (HTI) section. The successful candidate will be able to recruit at least two additional junior faculty members working on basic human immunology, patient-oriented clinical studies and on the translation of basic science discoveries to clinical therapies and/or diagnosis or prevention of disease. Additional individuals present at Yale could be incorporated into this program if this is desirable to the senior faculty recruit. The clinical target or targets of this program are likely to be complementary to ongoing activities within HTI and will be selected based on the interests and expertise of the recruit and on the resources and strengths of the Yale environment.

The application package must include a curriculum vitae, a summary of present and future research interests and three letters of recommendation. Completed applications should be received at the address below no later than **December 1, 2007**.

Jordan S. Pober, M.D., Ph.D
Chair, Search Committee
Department of Immunobiology
Yale University School of Medicine
P.O. Box 208089
New Haven, CT 06520-8089

An Equal Opportunity/Affirmative Action Employer.
We strongly encourage applications from women and minorities.

Baylor University

Chartered in 1845 by the Republic of Texas, Baylor University is the oldest university in Texas and the world's largest Baptist University. Baylor's mission is to educate men and women for worldwide leadership and service by integrating academic excellence and Christian commitment within a caring community. Baylor is actively recruiting new faculty with a strong commitment to the classroom and an equally strong commitment to discovering new knowledge as Baylor aspires to become a top tier research university all while retaining and remaining grounded in our strong Christian mission as described in Baylor 2012.

We invite you to come discover the vision (**www.baylor.edu/vision/**) and excitement at Baylor as we seek to fill the following **tenure-track** faculty position within the College of Arts and Sciences

DEPARTMENT CHAIR
DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY

Complete applications will include a current curriculum vitae, a description of research interests, a list of references, and a statement describing the candidate's interests and goals in seeking this position. Applications will be reviewed beginning **December 1, 2007**. To ensure full consideration an application should be received by **January 1, 2008**, but applications will be accepted until the position is filled.

Applicants should have a record of high quality research and demonstrated success in obtaining external funding. Previous administrative experience is desirable. Salary will be commensurate with experience and qualifications. Direct all correspondence to: **Bob Kane, Chair Search Committee, Department of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco, TX 76798**; E-mail: **Bob_Kane@baylor.edu**.

To learn more about the above position, the College of Arts and Sciences, and Baylor University, please visit **www.baylor.edu/chemistry/chairsearch** or **www.baylor.edu**.

Baylor is a Baptist university affiliated with the Baptist General Convention of Texas. As an Affirmative Action/Equal Employment Opportunity Employer, Baylor encourages minorities, women, veterans, and persons with disabilities to apply.

FACULTY POSITION IN Biophysics
UNIVERSITY OF COLORADO, BOULDER

The University of Colorado invites applications for a tenure-track faculty position in experimental biophysics, under the auspices of the interdisciplinary Initiative in Molecular Biotechnology (**http://bayes.colorado.edu/biotech**). Individuals with interests in biophysics, including but not limited to single-molecule biophysics, cellular biophysics, imaging, biological microfluidics, and biomaterials are encouraged to apply. Candidates at the **ASSISTANT, ASSOCIATE,** and **FULL PROFESSOR** levels will be considered. Candidates must have a Ph.D. degree and enthusiasm for teaching at undergraduate and graduate levels, and will be expected to develop an internationally recognized research program.

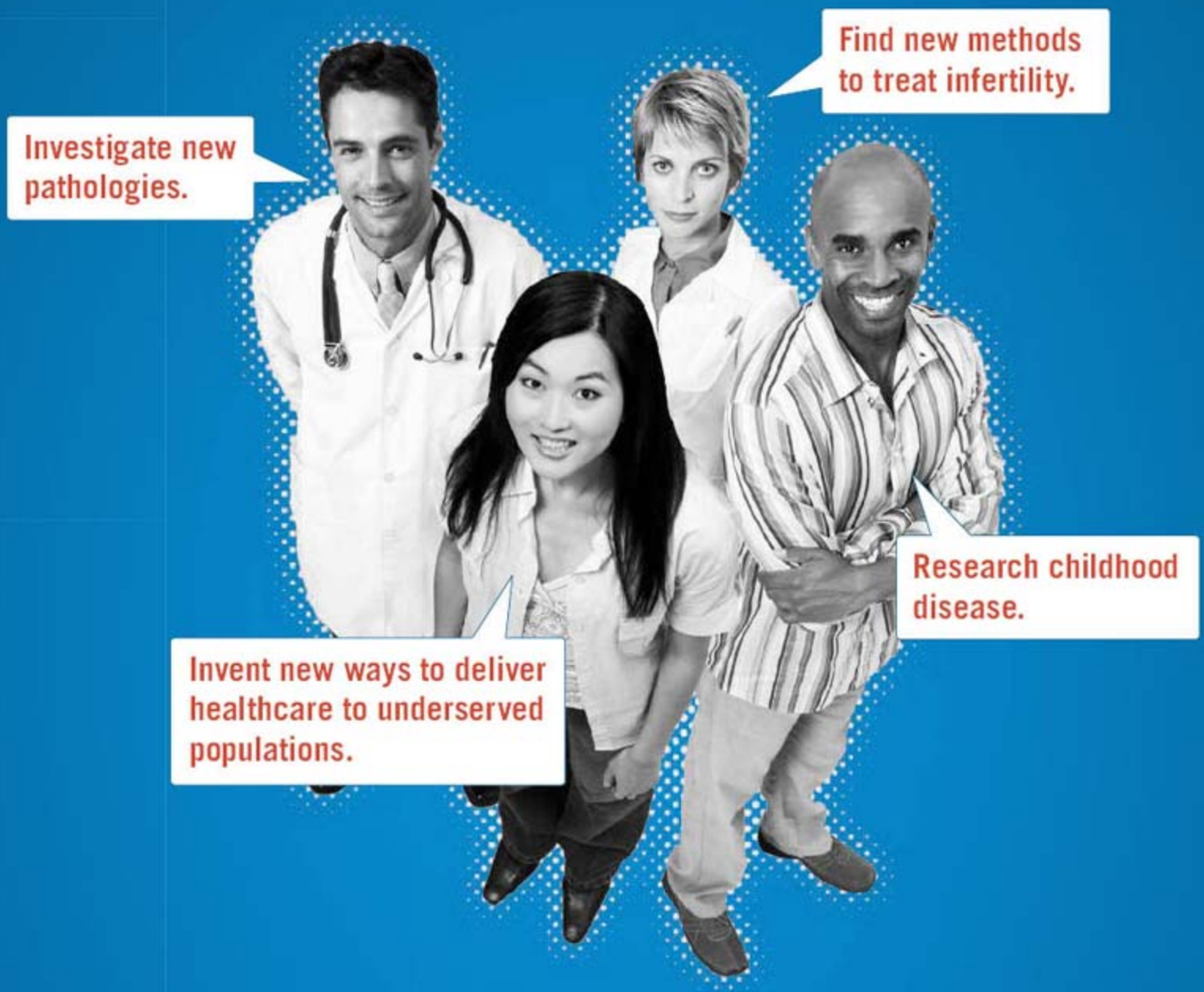
Applicants should submit a curriculum vitae, statements of research and teaching interests, and arrange to have three letters of reference sent to: **Biophysics Search Committee Chair, Campus Box 347, University of Colorado, Boulder, CO 80309-0347**. Electronic submission of all application materials may be sent to **BioTApp@colorado.edu**. For full consideration, applications should be received by **November 15, 2007**.

See **www.Colorado.edu/ArtsSciences/Jobs/** for full job description.

CU-Boulder is committed to diversity and equality in education and employment and is sensitive to the needs of dual-career couples.

IMAGINE.

Financial freedom. Following your dreams doing research that can change the world.



WE HEAR YOU. We can make it happen.

Secure a qualified research position at any non-profit medical center, university or community-based organization, and we'll repay your student loans.

THE NIH LOAN REPAYMENT PROGRAMS

Follow your passion. For eligibility requirements and to apply, go to www.lrp.nih.gov.

McLaughlin Research Institute
for Biomedical Sciences

PERIPHERAL MYELINATION/SCHWANN CELL DEVELOPMENT
Staff Scientist or Postdoctoral Scientist or Senior Research Assistant

An experienced scientist is sought as an active team member for a research project to identify genetic regulatory pathways that control axon-Schwann cell interactions and Schwann cell development. This position, in the laboratory of **John R. Bermingham, Jr.**, at the **McLaughlin Research Institute (http://www.montana.edu/wwwmri/)**, is supported by NINDS and private grants. Emphasis will be placed on developing Schwann cell-neuron coculture systems and on characterizing protein-protein interactions. The successful applicant will have a solid background in mammalian cell culture and protein biochemistry, and will be fluent in English. The position, either as a non-tenure track Staff Scientist (Ph.D), or a Postdoctoral Scientist, or an experienced Senior Research Assistant (BA/MS), will be filled by an applicant whose background best fits the project's needs. Salary is commensurate with experience.

McLaughlin Research Institute is a small, non-profit research organization located near **Montana's** Rocky Mountain front. Opportunities for outdoor recreation abound. Great Falls offers a pleasant environment, good public schools, and low housing costs. Applications, including names and contact information for three to five individuals who may serve as references, should be sent to:

John R. Bermingham, Jr., Ph.D.
McLaughlin Research Institute
1520 23rd Street South
Great Falls, MT 59405

PVLSI PIONEER VALLEY LIFE SCIENCES INSTITUTE

TWO NEW FACULTY POSITIONS

METABOLIC REGULATION/DIABETES

The Pioneer Valley Life Sciences Institute (PVLSI; **www.PVLSI.org**) is seeking a new **Faculty member (rank open)** who studies metabolic regulation and/or diabetes to join a collaborative research program of basic and clinical researchers. We welcome a variety of experimental approaches including: physiology, genetics and molecular biology. Expertise in mitochondria, cell death or thermoregulation particularly welcomed.

APOPTOSIS

The Pioneer Valley Life Sciences Institute (PVLSI; **www.PVLSI.org**) and the new Center of Excellence in Apoptosis Research (CEAR) are seeking a new **Faculty member (rank open)** who studies apoptosis with a focus on either disease or the development of new technologies. We welcome a variety of experimental approaches including physiology, genetics and molecular biology. Expertise in mitochondria, stem cells, metabolism, or drug delivery particularly welcomed.

Successful candidates will have an M.D. and/or Ph.D., post-doctoral experience, and depending on rank, a record of developing and maintaining an extramurally funded research program. Practicing physicians are encouraged to apply and can seek a clinical appointment in the appropriate department at the Baystate Medical Center, the western campus of Tufts University School of Medicine.

The PVLSI is an independent non-profit research organization that is jointly operated by the Baystate Medical Center (**www.Baystatehealth.com**) and the University of Massachusetts Amherst (**www.UMass.edu**). Researchers will have the opportunity to work closely with physicians and basic scientists in a newly built and well-equipped facility. Applicants should submit CV, statement of research plans, and three letters of reference to: **Dr. Lawrence Schwartz, PVLSI, 3601 Main Street Springfield, MA 01199** or via e-mail to **Lawrence.Schwartz@bhs.org** (PDF format preferred).

The PVLSI is an Equal Opportunity/Affirmative Action Employer.

UF | IFAS UNIVERSITY of FLORIDA

Director and Professor
Florida Sea Grant Program

The University of Florida is conducting a nationwide search for the Director and Professor, Florida Sea Grant Program. The Search Committee invites letters of nomination, applications (letter of interest, complete CV, and references), or expressions of interest to be submitted to the search firm assisting the University of Florida. Review of materials will begin immediately and continue until the appointment is made. It is preferred, however, that all nominations and applications be submitted prior to **December 20, 2007**. For a complete position description, refer to Current Opportunities on **www.parkersearch.com**.

Daniel F. Parker, Sr.
Laurie C. Wilder, Senior Vice President
770-804-1996 ext: 109
lwilder@parkersearch.com

The University of Florida is an Equal Opportunity, Affirmative Action Employer. Women and minorities are encouraged to apply. The "government in the sunshine" laws of Florida require that all documents relating to the search process, including letters of application/nomination and reference, be available for public inspection.

PARKER Executive Search
Five Concourse Parkway | Suite 2440 | Atlanta, GA 30328
770.804.1996 | parkersearch.com

BROWN

Department of Molecular Microbiology and Immunology
Brown University

FACULTY POSITION IN MICROBIOLOGY

A full-time faculty position is open for an Associate or Full Professor with tenure. Expertise in microbial pathogenesis. Preference will be given to applicants with a research focus complementing that of the existing faculty and the training program in Pathobiology of Infectious Disease and Host Defense. There is particular interest in work in bacterial systems. Final selection will be for a candidate of outstanding ability. Applicants must have a Ph.D. or M.D. degree, post-doctoral training and demonstrated research experience as an independent investigator. They must show evidence of ability to maintain a productive, independent, externally funded research program, and must demonstrate teaching excellence. Opportunities exist to interact with members of the Graduate and Postdoctoral Programs in the Division of Biology and Medicine. Academic advising of undergraduate and graduate students and service to the Division of Biology and Medicine are responsibilities assumed by all faculty members of the Department. The appointment will begin on or after July 1, 2008.

Applications received by **December 14, 2007** will receive full consideration. Applicants should submit a *curriculum vitae*, including research and teaching experience, publications, and research plans; enclose representative reprints, and have three letters of recommendation sent to:

Dr. Christine A. Biron,
Chairperson of the Faculty Search Committee
c/o Janina McEvoy, Department Manager
Department of Molecular Microbiology and Immunology
Box G-B6, Division of Biology and Medicine
Brown University, Providence, RI 02912

UNIVERSITY OF OKLAHOMA INTEGRATIVE LIFE SCIENCES INITIATIVE

A new frontier in Life Sciences awaits collaborative teams of chemists, biologists, mathematicians, and engineers who use advanced research tools to understand biological processes globally. The University of Oklahoma (Norman Campus) is continuing a multidepartment Integrative Life Sciences Initiative (ILSI) to complement and strengthen existing programs in botany and microbiology, chemistry and biochemistry, zoology, and bioengineering.

As part of this initiative the University invites applications for new tenured positions at the rank of Associate or Full Professor. We are seeking individuals with established world-class research programs in any of, **but not limited to**, the following general areas: developmental biology, neurobiology, neurochemistry, stem cells, genomics, proteomics, metabolomics, bioinformatics, cell signaling, cellular metabolism, bioenergetics, and structural biology.

Candidates must have a Ph.D. or equivalent terminal degree and a proven record of external grant funding. The successful individuals will be expected to contribute to undergraduate and graduate education in the life sciences, and provide leadership for the Integrative Life Sciences Initiative.

Applicants should submit a curriculum vitae, a description of their research plans, and a brief statement of their teaching interests and philosophy. Applicants should identify three individuals whom the Search Committee may contact for letters of recommendation. Application materials or nominations should be sent to: **Paul B. Bell, Jr., Dean of the College of Arts and Sciences and Vice Provost, Chair of the ILSI Search Committee, Ellison Hall Rm. 323, University of Oklahoma, 633 Elm Avenue, Norman, Oklahoma 73019**. We will also accept completed electronic applications in PDF format, sent to: **sbayliss@ou.edu**. Review of applications will begin on **December 1, 2007**, and continue until positions are filled.

Minorities and women are especially encouraged to apply. The University of Oklahoma is an Affirmative Action/Equal Opportunity Employer.

Post-doctoral Opportunities in Translational Bioinformatics at Stanford University

The laboratory of **Dr. Atul Butte** at Stanford Medical School is seeking highly motivated investigators to develop and study novel approaches in translational bioinformatics, or the application of analytic and interpretive methods to optimize the transformation of genome-scale data of many types into proactive, predictive, preventative, and participatory health. Positions are fully funded for 2 or more years.

Ideal candidates will have an M.D. or Ph.D. with a strong background in bioinformatics, biostatistics, and genomics, and a good publication record.

Strong problem-solving skills, quantitative and creative thinking, and the ability to build new software tools when needed, are required. Applicants must possess good communication skills and be fluent in both spoken and written English. A background in molecular biology, medicine, immunology, anesthesiology, or pharmacology will be a strong plus. Prior experience with genetic, microarray, proteomic, drug, or clinical databases is a plus.

This exciting work will be guided by multidisciplinary collaborations with top scientists in immunology, transplantation, and anesthesia research at Stanford. To apply, please send your CV, a brief statement of research interests, and contact information for three references to **Amy Erickson**, e-mail **amy.erickson@stanford.edu**, phone **(650) 725-3385**.

The Medical College of Georgia

The Department of Pathology at the Medical College of Georgia continues the expansion of its program in investigative pathology. We invite applications for two tenure-track faculty positions at the level of Assistant/Associate Professor engaged in basic and/or translational research related to cancer. Candidates must have completed productive postdoctoral training and developed original and externally fundable (for Assistant Professor) and funded (for Associate Professor) research programs related to the genesis, progression and prevention of cancer.

Areas of special interest include, but are not limited to, animal models of cancer, genetics/epigenetics of tumor cell biology, tumor-host microenvironment, and molecular control of cell proliferation and metastasis. The positions are supported by generous start-up package, and laboratories will be available in the new Cancer Center. The appointees will become part of a growing group investigating the role of G protein-coupled receptors and their effectors in tumor cell proliferation and metastasis.

Applicants should have MD and/or PhD degrees and significant postdoctoral experience.

Please send curriculum vitae including a statement of research interests and future plans, and the names of three references to: **Dr Yehia Daaka, Professor and Endowed Chair, Department of Pathology, c/o Mrs. Carol Hardy, Medical College of Georgia BF104, 1120 15th Street, Augusta, Georgia 30912** or **chardy@mcg.edu.**

The Medical College of Georgia is a Minority/Female/Veterans Equal Employment Opportunity, Affirmative Action, and Americans with Disabilities Act Employer.

THE HENRY SAMUELI SCHOOL OF ENGINEERING AT THE UNIVERSITY OF CALIFORNIA, IRVINE invites qualified applicants for three faculty positions in the **DEPARTMENT OF BIOMEDICAL ENGINEERING** beginning July 1, 2008. Two positions are at the rank of **Assistant Professor** (tenure track), and the other at the rank of **Associate Professor** (with tenure). Applicants must hold a Ph.D. degree in biomedical engineering or related field, and will be expected to maintain a broad-based extramurally funded research program. Of particular interest are candidates whose research program investigates the bioengineering and clinical aspects of biological systems including tissues and cells using techniques such as imaging, computational modeling, and MEMS. In addition, the successful candidate will be expected to advise students and teach undergraduate and graduate courses as well as develop collaborative programs with other faculty members and programs at UCI. The University of California, Irvine is situated in Orange County's rapidly growing high technology sector that includes more than 150 biomedical companies which are actively involved in our program.

APPLY NOW – submit your application to our on-line recruitment program.

For full consideration, candidates should upload applications electronically, please refer to the following website for instructions: **http://www.eng.uci.edu/employment/applicationinstructions**. Applications should include a curriculum vitae, a brief (no more than 2 pages) description of current and future research and teaching interests, and names of at least three references.

Questions regarding these positions may be addressed to **Ms. Radmila Milosavljevic radmila@uci.edu**. For more information about the Department of Biomedical Engineering please visit our website at **http://www.bme.uci.edu**. Applications will be accepted until the positions are filled, although maximum consideration will be given to applications received by **December 1, 2007**.

UCI is an Equal Opportunity Employer committed to excellence through diversity and strongly encourages applications from all qualified applicants, including women and minorities. UCI is responsive to the needs of dual career couples, is dedicated to work-life balance through an array of family-friendly policies, and is the recipient of an NSF ADVANCE Award for gender equity.

UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Postdoctoral Fellowships in Genomic Biology

The Institute for Genomic Biology at the University of Illinois offers a number of postdoctoral fellowships for talented young scholars. IGB Fellows spend two to three years doing collaborative research in one of several research themes at the Institute. Visit www.igb.uiuc.edu/fellows for more information about the Institute, the research themes, and application procedures. Closing date for all positions is January 15, 2008.

MOLECULAR BIOENGINEERING OF BIOMASS CONVERSION

We seek an individual with experience in metabolic engineering of industrially significant microbes (e.g. Yeast, fiber degrading bacteria, clostridia) for production of biofuels from biomass feedstocks. The ideal candidate will have expertise in modern molecular biology techniques, whole genome shuffling, DNA microarrays, proteomic and metabolic tools, transposon mutagenesis and high-throughput screening methods, to maximize the production of bioproducts and biofuels. (Hans Blaschek, Theme Leader)

PRECISION PROTEOMICS

We seek an individual to develop next-generation technologies at the intersection of mass spectrometry and chemical biology. The successful Fellow will have advanced training in mass spectrometry of proteins (with emphasis on "Top Down" techniques and tandem mass spectrometry of high-mass ions) associated proteomics software, capillary separations, and cell culture. The Fellow will work in an interdisciplinary fashion with expert labs in single molecule fluorescence, natural products discovery, and high-throughput screening for anti-cancer compounds. (Neil Kelleher, Theme Leader)

REGENERATIVE BIOLOGY AND TISSUE ENGINEERING

The Fellow will be responsible for developing protocols for quantifying gene expression profiles of both 2-D and 3-D cultural tissues and of cells excised from tissues in vivo. Additional responsibilities will include the development of models for investigations of embryonic and adult stem cell differentiation and development. The ideal candidate will have a strong background in embryology, stem cell biology and animal transgenesis. (Larry Schook, Theme Leader)

GENOMICS OF NEURAL AND BEHAVIORAL PLASTICITY

We seek a biologist with training in one or more of the following areas: evolutionary biology, neuroscience, animal behavior, molecular biology, genomics, and bioinformatics. The successful candidate will join a multi-disciplinary team that is using genomics to identify both conserved and novel mechanisms of neural and behavioral plasticity in diverse animal systems. Fellows are expected to conduct research that contributes to the development of the theme's goals by integrating components from theme member's individual research programs. (Gene Robinson, Theme Leader)

BIOCOMPLEXITY

We seek a quantitative scientist with interests in evolution, systems biology and ecosystem dynamics, and expertise in statistical physics as applied to biology. The successful candidate will join a multi-disciplinary group exploring collective effects in biology. Projects include the evolution of translation, the role of horizontal gene transfer in communities of microbes and phages, and the systems biology of cells and ecosystems. (Nigel Goldenfeld, Theme Leader)

MINING MICROBIAL GENOMES FOR NOVEL ANTIBIOTICS

The Fellow will be involved in a multi-disciplinary project aimed at the discovery, design, and development of phosphonic acid antibiotics. The ideal candidate will have a proven record of expertise in the general area of microbially produced natural products, with specific expertise in one of several disciplines. We are interested in candidates with previous experience in bacterial metabolism, bacterial genetics, molecular biology, biochemistry, enzyme evolution, organic synthesis, mass spectroscopy, bioinformatics and metagenomics. (Bill Metcalf, Theme Leader)

GENOMIC ECOLOGY OF GLOBAL CHANGE

The Fellow will be involved in a cross-disciplinary project investigating how changes in networks of genes affect ecosystem metabolism when challenged by elements of global change, including elevated atmospheric carbon dioxide and ozone, increased drought, and altered interactions with insect herbivores and plant pathogens. The ideal candidate will have a strong background in plant biology and a record of expertise in molecular biology, genomic ecology, physiology or modeling of gene networks or ecosystem function. The ability to work creatively and productively in a highly interdisciplinary and collaborative environment is essential. (Don Ort, Theme Leader)

HOST-MICROBE SYSTEMS

The Fellow will be responsible for developing DNA isolation and 16S rRNA and other ribotyping and metagenomic library construction techniques for surveying microbial content of the vagina. Additional responsibilities will include the development of microarray and other molecular biology techniques to examine vaginal contents, and performing analyses using bioinformatics and other computational and analytical methods. The ideal candidate will have a strong background in microbiology, biochemistry, chemistry, or a related field with experience and expertise in molecular microbial ecology and bioinformatics. (Brenda Wilson, Theme Leader)

BUSINESS, ECONOMICS, AND LAW OF GENOMIC BIOLOGY

We seek an individual with training in economics, business, law, or strategy and with an interest in technology entrepreneurship, technology industries, and biotechnology. The Fellow will join a multi-disciplinary group that includes business, law, technology experts, and agricultural economics faculty, and personnel from the campus Office of Technology Management. Our theme is exploring issues in university-industry technology transfer, industry evolution, intellectual property protection, the competitive and cooperative dynamics for both entrepreneurial start-ups and existing corporations, the impact that globalization of biotechnology has on the evolution of industry, and the position of U.S. firms in the global marketplace. (Jay Kesan, Theme Leader).

WWW.IGB.UIUC.EDU

TENURE-TRACK POSITION
DIRECTOR OF MEMORY DISORDERS PROGRAM
DEPARTMENT OF NEUROLOGY
GEORGETOWN UNIVERSITY MEDICAL CENTER

The Department of Neurology at Georgetown University Medical Center invites applications for a tenure-track position at the Associate or Full Professor level. The successful candidate will be Board-certified in Neurology or a related discipline, will have a track record of independently funded research in dementia, and will have strong leadership skills. This position will entail responsibility for leading the Memory Disorders Program, an active, well-established clinical research program in Alzheimer's disease and other dementias, which includes both NIH- and industry-sponsored therapeutic trials. The successful candidate will also supervise the clinical services of the Memory Disorders Program, participate in the teaching activities of the Department of Neurology and the Interdisciplinary Program in Neuroscience, and conduct his/her own research program.

Inquiries, including a current curriculum vitae and letter of interest, should be directed to:

Rhonda B. Friedman, Ph.D.
Chair, Search Committee for MDP Director
Department of Neurology, Georgetown University
207 Bldg D
4000 Reservoir Rd. NW
Washington, D.C. 20057
friedmar@georgetown.edu

Georgetown University is an Equal Opportunity/Affirmative Action Employer with a strong institutional commitment to the achievement of excellence of diversity among its faculty and staff.

FACULTY POSITIONS
IMAGING AND CELL BIOLOGY
THE GRUSS-LIPPER BIOPHOTONICS CENTER

Innovative and creative scientists are invited to apply for faculty positions at any level in the Gruss-Lipper Biophotonics Center of the Albert Einstein College of Medicine. Members will be tenured, or tenure-track faculty in the Department of Anatomy and Structural Biology. Candidates are expected to have a background in any of the following: Biophysics, Physics, Electrical Engineering, Biology or Chemistry, but with a research focus in microscopy and imaging as related to the cell biology of human disease.

Core facilities will include chemical genomics, bioinformatics and computational biology, human genetics, microarray and sequencing, protein chemistry and proteomics, gene therapy and transgenic mice. The Gruss-Lipper Biophotonics Center will also contain an Innovation Laboratory including a microscope fabrication facility, laser workshop, a multiphoton microscope, rapid live cell imaging microscope, single molecule detection, and optical and software engineering support. The Biophotonics Center also maintains a service component, the Analytical Imaging Facility, which includes comprehensive light, electron and cryo-electron microscopy services.

Please e-mail letter of introduction, curriculum vitae, research plan and three letters of recommendation to: **Gruss-Lipper Biophotonics Search Committee, c/o Maritza Reyes, Administrator, Albert Einstein College of Medicine, Jack & Pearl Resnick Campus, 1300 Morris Park Avenue, Forchheimer 620, Bronx, NY 10461; email: mreyes@aecom.yu.edu**

We welcome applicants who will add diversity to our academic leadership and faculty. Equal Opportunity Employer.

ALBERT EINSTEIN COLLEGE OF MEDICINE OF YESHIVA UNIVERSITY

JOHNS HOPKINS
MEDICINE
SCHOOL OF MEDICINE

Faculty Positions
The Solomon H. Snyder
DEPARTMENT OF NEUROSCIENCE

Applications are invited for tenure-track faculty positions at both junior and senior levels in the Solomon H. Snyder Department of Neuroscience at the Johns Hopkins University School of Medicine. Applicants should have interests in molecular, cellular, developmental, systems or behavioral neuroscience, a Ph.D. or M.D., and a strong record of research accomplishments. Faculty members are expected to have, or establish, creative independent research programs and participate in teaching graduate and medical students. Deadline for applications is **November 30, 2007**. The Johns Hopkins University is committed to enhancing the diversity of its faculty and encourages applications from women and minorities.

Please submit a PDF file containing curriculum vitae, names and contacts for three references and a brief description of current and future research interests.

Richard L. Huganir, Ph.D.
Search Committee
Department of Neuroscience
The Johns Hopkins University
School of Medicine
725 North Wolfe Street, PCTB 904
Baltimore, Maryland 21205
JHUNeuroscience@jhmi.edu

An EEO/AA Employer.

Ara G. Paul Professor
and
Ara G. Paul Assistant Professor
Department of Pharmaceutical Sciences
The University of Michigan College of Pharmacy

The Department of Pharmaceutical Sciences is entering a new and exciting phase in its growth and development, and invites applications for two Endowed Faculty Professorships. At the senior Professor level, we are looking for a tenured scientist with outstanding leadership and research credentials, and a demonstrated record of accomplishment in securing extramural funding. At the junior Assistant Professor level, we are looking for a tenure-track scientist with the potential for outstanding scholarly activity in pharmaceutical sciences research and education. Both positions demand a faculty member that is committed to professional and graduate education, and to professional service.

Applicants who are performing high-impact research in the following areas of interest, as applied to the pharmaceutical sciences, are preferred: (1) cellular and molecular drug delivery and targeting, (2) biomaterials and nanotechnology (3) drug transport and/or metabolic mechanisms, (4) structural biology, (5) cheminformatics, and (6) pharmacogenomics.

Interested individuals should send a letter of application, curriculum vitae, future research plans and contact information of three references (i.e., name, address, telephone and email) to the Search Committee Chair via US Mail. A PDF file should also be sent to **tjasin@umich.edu** as an email attachment. The Search Committee will begin reviewing applications immediately and will continue to do so until the positions are filled. Submit the requested information to: **Professor Victor C. M. Yang, Search Committee Chair, College of Pharmacy, The University of Michigan, 428 Church Street, Ann Arbor, MI 48109-1065**. For more information, please contact the department at: **Telephone: 734-615-3749; Facsimile: 734-615-6162; E-mail: tjasin@umich.edu**.

The University of Michigan is an Equal Opportunity/ Affirmative Action Employer.

DEPARTMENT OF COMMUNICATIONS, ENERGY AND NATURAL RESOURCES
ROINN CUMARSÁIDE, FUINNIMH AGUS ACMHAINNÍ NÁDÚRTHA

THE DEPARTMENT OF AGRICULTURE & FOOD
AN ROINN TALMHAÍOCHTA AGUS BIA

BEAUFORT MARINE RESEARCH AWARDS

- **4 Principal Investigators (7 year Contracts)**
- **15 Researcher Posts (7 year Contracts)**
- **28 PhD Studentships (4 year Stipend)**

The Irish government has launched a major new strategy to maximise the scientific, economic and social potential of Ireland's significant marine resources. **Sea Change: A Marine Knowledge, Research and Innovation Strategy for Ireland 2007-2013** will support a range of capability and capacity enhancements designed to exploit core scientific strengths and deliver new commercial opportunities and policy options. As part of this strategy the recently announced **Beaufort Marine Research Awards** will deliver €20 million in research funds to establish world class marine research groups in five areas. The Beaufort Awards target research leaders of international standing and mobile early stage researchers from Ireland and abroad.

The Minister for Communications, Energy & Natural Resources and the Minister for Agriculture, Fisheries & Food recently announced the 10 Research Groups/Centres that were successful in their bid for the Awards, following evaluation by international experts. The research groups/centres are located in five Institutions on the Island of Ireland (32 counties) and have demonstrated capacity to conduct innovative research and provide research training. The following are the research groups/centres supported:

Ecosystem Approach to Fisheries Management

- **Ecology & Evolution Research Cluster, School of Biological Sciences, Queen's University Belfast**
  Contact: Dr. Keith Farnsworth Email: k.farnsworth@qub.ac.uk Tel: + 44 28 9097 2352
  http://www.qub.ac.uk/bb/research-clusters/ecology-evolutionary.html
  Research Group requires – 1 Researcher ; 2 PhD Students
- **Marine Institute**
  Contact: Dr. Paul Connolly Email: paul.connolly@marine.ie Tel: + 353 91 387200 http://www.marine.ie
  Research Group requires – 1 Principal Investigator
- **Aquaculture & Fisheries Development Centre, University College Cork**
  Contact: Prof. Gavin Burnell Email: g.burnell@ucc.ie Tel: + 353 21 4904650
  http://www.ucc.ie/en/DepartmentsCentresandUnits/AquacultureFisheriesDevelopmentCentre/
  Research Group requires – 2 Researchers; 2 PhD Students.

Marine Biodiscovery

- **Environmental Research Institute & Microbiology Department, University College Cork**
  Contact: Prof. Alan Dobson Email: a.dobson@ucc.ie Tel: + 353 21 4901946 http://www.ucc.ie/en/ERI/
  Research Group requires – 2 Researchers; 4 PhD Students.
- **Martin Ryan Institute, National University of Ireland, Galway**
  Contact: Prof. Michael Guiry Email: mike.guiry@nuigalway.ie Tel: + 353 91 492339 http://mri.nuigalway.ie
  Research Group requires – 1 Principal Investigator; 2 Researchers, 4 PhD Students.
- **Marine Biodiscovery Consortium, School Of Biological Sciences, Queen's University Belfast**
  Contact: Dr Mark Johnson Email: m.johnson@qub.ac.uk Tel: + 44 28 9097 2297 http://www.qub.ac.uk/bb/
  Research Group requires – 2 Researchers; 4 PhD Students.

Marine Sensors & Communications

- **National Centre for Sensor Research, Dublin City University**
  Contact: Prof. Dermot Diamond Email: dermot.diamond@dcu.ie Tel: + 353 1 7005404 http://www.ncsr.ie
  Research Group requires – 1 Principal Investigator; 1 Researcher; 5 PhD Students.

Fish Population Genetics

- **Department of Zoology, Ecology & Plant Science, University College Cork**
  Contact: Prof. Tom Cross Email: t.cross@ucc.ie Tel: + 353 21 4904652 http://www.ucc.ie/academic/zeps/
  Research Group requires – 1 Principal Investigator; 2 Researchers; 1 PhD Student.
- **Fisheries Genetics & Molecular Ecology Research Group & Fisheries & Aquatic Ecosystems Branch, Queen's University Belfast**
  Contact: Dr Paulo Prodohl Email: p.prodohl@qub.ac.uk Tel: +44 2890972267 http://www.qub.ac.uk/bb/
  Research Group requires – 1 Researcher; 2 PhD Students.

Marine Economic & Social Research

- **Irish Centre for Rural Transformation & Sustainability (ICERTS), National University of Ireland, Galway**
  Contact: Prof. Michael Cuddy Email: michael.cuddy@nuigalway.ie Tel: + 353 91 750324
  http://www.nuigalway.ie/icerts/
  Research Group requires – 2 Researchers; 4 PhD Students.

The research groups/centres above will be advertising shortly for the following positions:

PRINCIPAL INVESTIGATORS – Up to seven years funding on salary scale €80,177 pa to €110,000 pa. PhD and a minimum of seven years post-doctoral research experience required.

RESEARCHERS – Up to seven years funding on salary scale €55,000 pa to €80,500 pa for researchers who have obtained a PhD and have minimum of 3 years postdoctoral research experience.

PhD STUDENTSHIP – 4 year stipend. €18,000 pa plus tuition fees. As part of the PhD studentship funding is available for stays in international centres of excellence.

Interested candidates should contact individual research groups/centres directly for further information on the application process.

NDP
National Development Plan 2007 - 2013
Transforming Ireland

Queen's University Belfast

UCC
Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

SeaChange
Casadh na Taoide

DCU

National University of Ireland, Galway
Ollscoil na hÉireann, Gaillimh

Marine Institute
Fóras na Mara

Director of the Indiana University Cyclotron Facility

Applications are invited for the directorship of the Indiana University Cyclotron Facility (IUCF). The IUCF has a diverse mission of multi-disciplinary research, service and education and also provides technical support and beam to the Midwest Proton Radiotherapy Institute (MPRI), one of only five such facilities in the United States.

We seek a scientist or a science manager with a distinguished record of accomplishment who will provide the vision, leadership, and entrepreneurial spirit needed to sustain and further IUCF's outstanding scientific research efforts, and will support the partnership with MPRI in the development of new radiotherapy treatments, technologies and applications.

IUCF conducts research in a variety of areas, including accelerator physics, condensed matter physics, medical physics, nuclear physics, nuclear chemistry, radiation effects on materials and radiation biology. The facility collaborates closely with the nationally ranked nuclear physics program at Indiana University Bloomington, which is currently developing detectors for use at national facilities to study high-energy spin physics, fundamental neutron physics, and neutrino oscillations. The facility is also developing novel neutron scattering instrumentation for the Low-Energy Neutron Source at IUCF, which recently began operation.

The director will serve a five-year term starting in the summer of 2008 and will receive a tenured professorship in the College of Arts and Sciences. Salary will be commensurate with experience and qualifications. Nominations are welcome. Applications with a complete resume, including the names of four references, should be sent as soon as possible to: **Professor Alex Dzierba, Chairperson, Search and Screen Committee, Office of the Vice Provost for Research, Indiana University, Franklin Hall 116-Y, Bloomington, IN 47405-1223**. Or email applications to: **DLTAYLOR@indiana.edu**.

Indiana University is an Affirmative Action/Equal Opportunity Employer, and encourages applications from women and minority candidates.

Tulane University

Tulane University School of Medicine Immunology Program Leader

Tulane University School of Medicine is undergoing a period of unprecedented expansion and renewal and will be hiring new faculty at all ranks in areas of strategic research emphasis. As the first step in this initiative, Tulane University School of Medicine is seeking an exceptional and visionary leader to develop a comprehensive, independent program in basic and translational research in Immunology. The leader of the Immunology research initiative will be provided the resources to recruit additional investigators whose research complements existing strengths and renewed emphasis in Cancer, Cardiovascular Disease/Hypertension, Infectious Diseases, Gene Therapy, Lung Biology, Neuroscience, or Solid Organ Transplantation. These individuals will join a growing and interactive group of researchers in both the basic and clinical departments at the School of Medicine (**http://www.som.tulane.edu/**). Applicant must possess an M.D. or Ph.D. or equivalent, and have an established and internationally recognized research program in one of these program areas.

This position also provides an opportunity for interdisciplinary research with faculty at the Tulane National Primate Research Center (**http://www.tnprc.tulane.edu/index.shtml**), Tulane University School of Public Health and Tropical Medicine (**http://www.sph.tulane.edu/**), and Tulane University School of Science and Engineering (**http://www.sse.tulane.edu/**). Additional information about Tulane University and the New Orleans area may be obtained on the Tulane University web site (**http://tulane.edu/**).

Applicants should submit a curriculum vitae and statement of research interests and goals to: **Dr. John Clements, Vice Dean for Research, Tulane University School of Medicine - SL38, 1430 Tulane Avenue, New Orleans, LA 70112**. Completed applications may be sent by e-mail to **research@tulane.edu**. Review of applications will remain open until the position is filled.

Tulane University is an Equal Opportunity, Affirmative Action Employer and encourages applications from minorities, women, and other qualified persons.

SANFORD SCHOOL OF MEDICINE

Assistant Professor in Immunology

The Division of Basic Biomedical Sciences at the Sanford School of Medicine of The University of South Dakota invites applications for a tenure-track faculty position at the Assistant Professor level. Exceptional applicants at higher levels may also be considered. Applicants should have a Ph.D. and/or M.D., or equivalent degree, and post-doctoral experience. Successful candidates will be expected to develop an independent, externally funded research program in the field of immunology, particularly as it relates to response to infectious diseases and complements existing strengths in the Division. Division faculty, along with colleagues at several other regional institutions, have developed a strong research consortium focused on the molecular pathogenesis of Gram-positive infectious agents. Information on the Midwest Consortium on Gram-positive Pathogenesis (MCGP) can be found at **http://www.ld-interactive.com/clients/grampositive/**

The successful candidate also will be expected to participate in teaching undergraduate, graduate, and medical students. Excellent start-up funds, state-funded salary commensurate with experience and modern research facilities in the new Lee Medical Building in Vermillion, SD will be provided. The Division of Basic Biomedical Sciences unites the classic basic science medical departments into a single administrative unit; a structure that breaks down traditional boundaries and allows interdisciplinary collaboration to flourish. In addition to infectious diseases other areas of research strength include cardiovascular diseases, oncology, cell and molecular regulation, neuroscience and protein quality control. Shared, state of the art core facilities are also available.

Applicants should include curriculum vitae, representative reprints, summary of past experience, statement regarding research interests and future plans, as well as three letters of recommendation. All materials should be sent to The University of South Dakota online employment website at **http://yourfuture.sdbor.edu** Review of applications will begin Nov. 30, 2007 and continue until position is filled. AA/EOE

SANFORD SCHOOL OF MEDICINE
The University of South Dakota.

University of Michigan
Center for stem cell biology
life sciences institute

The Life Sciences Institute and the University of Michigan Medical School invite applications for tenure track **ASSISTANT PROFESSOR** positions. We are seeking outstanding scholars, with Ph.D., M.D. or equivalent degrees and relevant postdoctoral experience, who show exceptional potential to develop an independent research program that will address fundamental issues in any aspect of stem cell biology. Applicants who have already established successful independent research programs will be considered for tenured **ASSOCIATE PROFESSOR** or **PROFESSOR** positions.

Applicants should send a curriculum vitae, copies of up to three reprints, a one- to two-page summary of research plans, and arrange to have three letters of reference sent directly by **November 1, 2007** to:

Stem Cell Search Committee
c/o Rebecca Fritts
Life Sciences Institute
University of Michigan
210 Washtenaw Avenue
Ann Arbor, Michigan, 48109-2216

The University of Michigan is an Affirmative Action/Equal Opportunity Employer.

Plant Science Innovation at the University of Nebraska-Lincoln

The Plant Science Community at the University of Nebraska-Lincoln is a vibrant, rapidly growing group of scientists focused in research areas that include chromatin biology, abiotic and biotic stress responses, organelle biology, metabolic biochemistry, and genetic engineering. We wish to announce several important upcoming opportunities in plant science at UNL:

Plant Comparative Genomics Position

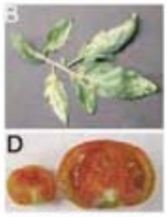
A joint, tenure-track Assistant or Associate Professor position is currently available in the Plant Science Initiative (**http://psiweb.unl.edu**) at the University of Nebraska-Lincoln. The position is 80% research/20% teaching with an academic home in the Department of Agronomy and Horticulture. The successful candidate is expected to maintain a vigorous, externally funded research program focused on comparative genomics of plants. Strong preference will be given to research programs that focus on crop genome evolution, computational biology, large scale cross-genome comparison or DNA marker-based analysis, or related areas. Teaching responsibilities include teaching one graduate or undergraduate level course annually in Plant Systematics, and mentoring students. A Ph.D. and post-doctoral experience in plant genetics, plant biology or systematics, computational biology or related field is required. Salary is commensurate with qualifications and experience. Review of applications will begin **November 30, 2007**, and continue until the position is filled or the search is closed. Applicants should go to **http://employment.unl.edu/** (requisition **#070778**) and complete the Faculty/Administrative Information form and then send a complete application file consisting of a statement of research interests, a current CV and arrange for three letters of recommendation to: **Search Committee Chair, PSI Comparative Genomics Position, N300 Beadle Center for Genetics Research, University of Nebraska-Lincoln 68588-0660 (USA)**.

Plant Epigenetics Position

A joint, tenure-track Assistant or Associate Professor position is currently available in the Plant Science Initiative (**http://psiweb.unl.edu**) at the University of Nebraska-Lincoln. The position is 80% research/20% teaching with an academic home in the School of Biological Sciences. The successful candidate is expected to maintain a vigorous, externally funded research program focused on epigenetic biology of plants. Strong preference will be given to research programs that focus on RNA interference, non-coding RNAs, chromatin structure and function, genome-wide analyses of histone or DNA modifications, gene silencing, paramutation or genomic imprinting. Teaching responsibilities include teaching one graduate or undergraduate level course annually in a relevant area, and mentoring students. A Ph.D. and post-doctoral experience in genetics, molecular biology, systems biology or a related field is required. Salary is commensurate with qualifications and experience. Review of applications will begin **November 30, 2007**, and continue until the position is filled or the search is closed. Applicants should go to **http://employment.unl.edu/** (requisition **#070789**) and complete the Faculty/Administrative Information form and then send a complete application file consisting of a statement of research interests, a current CV and arrange for three letters of recommendation to: **Search Committee Chair, PSI Epigenetics Position, N300 Beadle Center for Genetics Research, University of Nebraska-Lincoln 68588-0660 (USA)**.

Graduate Traineeships in Plant Systems Biology and Molecular Plant Breeding. See: **http://plantsciences.unl.edu**

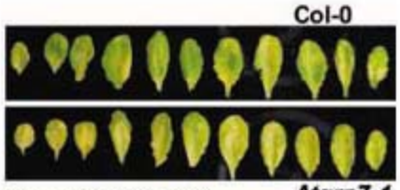
PNAS **104:**1766, 2007



Inducible male sterility

Science **25:**316, 2007

Dicamba resistant crops



Nature **447:**284, 2007

Type III secretion in plant disease

Postdoctoral Positions

The Plant Science Initiative (**http://psiweb.unl.edu**) invites applications for two postdoctoral research associate positions. The successful candidates will contribute to studying the role of transcription factors and histone modifications in the responses of Arabidopsis and corn to water. We are particularly interested in characterizing chromatin modifications associated with stress-responsive genes as well as the target network of a subset of inducible transcription factors. Genome-wide approaches, such as chromatin immunoprecipitation and high throughput sequencing, will be used to analyze transcription factor binding and histone modifications during water stress. The research environment includes state-of-the-art facilities in the George W. Beadle Center for Genetics and Biomaterials Research and opportunities in genomics, proteomics, and bioinformatics analyses. A Ph.D. degree in Plant Biology, Biological Sciences, Genetics, or a related field is required. Experience in molecular genetics, protein biochemistry, and/or antibody production is highly desirable. Review of applications will begin **November 23, 2007** and continue until suitable candidates are found. Interested candidates should complete the Faculty/Administrative application form at **http://employment.unl.edu/** (requisition **#060042**) and then send a cover letter, current CV and the names and contact information of three references to **Dr. Heriberto Cerutti (hcerutti1@unl.edu)** or **Dr. Michael Fromm (mfromm2@unl.edu) E205/E211 Beadle Center, P.O. Box 880666, University of Nebraska-Lincoln, Lincoln, NE 68588 USA**.

For more see: **http://www.plantsciences.unl.edu/**

UNL is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity. We assure reasonable accommodation under the Americans with Disabilities Act. Contact Barbara Gnirk at (402) 472-2635 or bgnirk1@unl.edu for assistance.

Université McGill University
Faculty of Medicine/Faculté de médecine

McGill

Chair
Department of Anatomy and Cell Biology

The Faculty of Medicine at McGill University, one of the oldest and most respected in North America, is inviting applications as part of an international search for the position of Chair, Department of Anatomy and Cell Biology.

The Department of Anatomy and Cell Biology has a strong tradition of excellence in research and teaching. It consists of 16 full-time academic staff members, 4 part-time appointees, 11 adjunct professors and 12 associate members and is well supported by CIHR, NSERC, NIH and other funding agencies. It offers a dynamic research environment with extensive inter-departmental and multi-disciplinary research collaborations. Opportunities exist to develop existing areas and build new areas of strength through recruitment. The Department participates in teaching at all levels including undergraduate, graduate and medical students; and postdoctoral fellows. Further details can be found at: **http://www.mcgill.ca/anatomy/**.

Candidates should have a commitment to research and teaching and an international reputation in the field of anatomy and cell biology broadly defined at the systems, cellular or molecular levels is essential. The successful applicant will have completed a doctoral degree and have a strong record of research accomplishments and proven administrative and teaching skills.

Interested applicants should email their curriculum vitae, including a list of publications, a statement of interest, as well as the names, addresses and emails of three references. Please indicate **'Chair, Anatomy and Cell Biology'** in the subject line. The address is: **facultyaffairs.med@mcgill.ca**; **c/o Dr. John A. Robson, Faculty of Medicine, McGill University, 3605 de la Montagne, Montreal, QC H3G 2M1, CANADA.** The deadline for submission is **January 31, 2008**.

Candidates would benefit from a working knowledge of both official languages. All qualified candidates are encouraged to apply, however, in accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. McGill University is committed to equity in employment.

Université McGill University
Faculty of Medicine/Faculté de médecine

McGill

Chair
Department of Psychiatry

The Faculty of Medicine at McGill University, one of the oldest and most respected in North America, is inviting applications as part of an international search for the position of Chair, Department of Psychiatry.

The Department of Psychiatry has a strong tradition of excellence in research and teaching. It consists of 143 full-time academic staff members, 30 part-time appointees, 17 adjunct professors and 35 associate members and is well supported by CIHR, NSERC, NIH and other funding agencies. It offers a dynamic research environment with extensive inter-departmental and multi-disciplinary research collaborations. Opportunities exist to develop existing areas and build new areas of strength through recruitment. The Department participates in teaching at all levels including undergraduate, graduate and medical students, medical residents and postdoctoral and clinical fellows. Further details can be found at: **http://www.mcgill.ca/psychiatry/**.

Applicants should have senior academic experience with proven administrative and teaching skills. A commitment to research with an international reputation in this domain is an important attribute. The selected candidate must be a M.D. and be licensed, or eligible for licensure, in the Province of Quebec.

Interested applicants should email their curriculum vitae, including a list of publications and a statement of interest, along with the names, addresses and emails of three references. Please indicate **'Chair, Psychiatry'** in the subject line. The address is: **facultyaffairs.med@mcgill.ca**; **c/o Dr. John A. Robson, Faculty of Medicine, McGill University, 3605 de la Montagne, Montreal, QC H3G 2M1, Canada.** The deadline for submission is **January 31, 2008**.

Candidates would benefit from a working knowledge of both official languages. All qualified candidates are encouraged to apply, however, in accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. McGill University is committed to equity in employment.

Temple University School of Medicine (TUSM) invites applications for tenure-track faculty positions in the Department of Physiology at the Assistant, Associate or full Professor levels. The Department, in collaboration with the Cardiovascular Research Center, is seeking new faculty whose expertise is in cardiovascular biology. Desired areas of research emphasis include mechanisms of cardiovascular injury and repair, and cardiac regeneration. Faculty will be expected to have or to develop independent, extramurally funded research programs. Requirements: Ph.D., M.D. or equivalent degrees and post-doctoral research experience;

Salary and rank commensurate with qualifications and experience.

Candidates are asked to send their applications (including curriculum vitae, a concise summary of their research program and names and contact information for three references) to: **Dr. Steven Houser, Chair, Department of Physiology and Director, Cardiovascular Research Center, Temple University School of Medicine, 3400 North Broad Street, Philadelphia, PA 19140** (or electronically to **patricia.parker@temple.edu**)

Temple University is an Affirmative Action/ Equal Opportunity Employer and strongly encourages applications from women and minorities.

Department Chair and Professor Chemistry and Biochemistry Florida Atlantic University

The Department of Chemistry and Biochemistry in the Charles E. Schmidt College of Science at FAU located in Boca Raton, Florida, invites applications at the full professor level from distinguished researchers for the position of department chair. The chair will be expected to provide dynamic leadership as a researcher, mounting an internationally visible research program at FAU, while also overseeing operations and development of the department. Applicants from all areas of chemistry and biochemistry are invited, especially those complementing research programs of the current faculty, and will have a record of significant external funding with currently active grants. Our dynamic and growing department is comprised of a cohesive group of externally funded researchers overseeing more than 40 doctoral students. For more information on the department and its programs, please visit **http://www.science.fau.edu/chemistry**. Applicants must apply online at **http://jobs.fau.edu/** and attach to their application a curriculum vitae, descriptions of current research and external funding, and the names and addresses of at least three references. A background check will be required for the candidate selected for this position. Materials received by **January 4, 2008** will receive full consideration; however, this position will remain open until filled. Questions or statements of initial interest about this position can be sent by e-mail to **Prof. Gregg Fields**, Chair, Dept. of Chemistry and Biochemistry (**fieldsg@fau.edu**). A state-supported institution with over 25,000 students, *FAU is an Equal Opportunity Employer/Equal Access Institution.*

COLUMBIA UNIVERSITY
Assistant Professor
(Tenure track)

The Department of Biochemistry and Molecular Biophysics (DBMB) seeks to hire an Assistant Professor (Tenure track). We are interested in persons studying the function of macromolecules using a combination of in vivo, biochemical, and/or molecular biological approaches. DBMB fosters a multidisciplinary environment that integrates biological structure with function through highly interactive collaborations between individual faculty members.

Additional information about DBMB is available at **http://cpmcnet.columbia.edu/dept/gsas/biochem/**.

Applications should include a CV, reprints of no more than three publications, and a brief statement of future research goals; three or more letters of reference should be sent independently. Send application material by **December 15, 2007** to: **Search Committee, Biochemistry and Molecular Biophysics, Box 36, 630 West 168th Street, New York, NY 10032**.

We take Affirmative Action toward Equal Employment Opportunity.

University of Pittsburgh

University of Pittsburgh Center for HIV Protein Interactions (PCHPI)

The University of Pittsburgh has received a five-year grant from the National Institute of General Medical Sciences to establish an interdisciplinary center that will bring scientists and facilities of the highest caliber together with the goal of elucidating the interactions of HIV proteins with host cell factors and will provide methodologies and tools to the HIV research community at large. The PCHPI seeks to provide insight into an important area of HIV pathogenesis and open doors for exploring and developing alternative anti-HIV strategies. The Principal Investigator and Director of the Center is **Angela Gronenborn, Ph.D.**, Professor and Chair of the Department of Structural Biology at the University of Pittsburgh. The Co-Director of the Center is **Ronald Montelaro, Ph.D.**, Professor, Department of Molecular Genetics and Biochemistry, University of Pittsburgh. Please visit our website at **http://www2.structbio.pitt.edu/hivppi/site/**.

Qualified candidates are being sought for the following positions:

POSTDOCTORAL ASSOCIATE – CRYO-ELECTRON TOMOGRAPHY

Will develop cryo-electron tomography of mammalian cells and study virus-host cell interaction. Dept. of Structural Biology is equipped with state-of-the-art EM equipment for cryo-EM studies, including a FEI Polara 300kv helium stage with a high-resolution 4kx4k CCD camera, a TF20 FEG with 4kx4k CCD camera, a T12spirit, and an FEI vitrobot for specimen preparation. Qualifications include a Ph.D. and an interest in the structural characterization of complex biological systems. Experience in cryo-transmission electron microscopy, image processing, and cell biology is desirable. Experience in vitreous section is a plus. Interested candidates can directly send a CV including areas of expertise and interest, publications list, and names and contact information of three references to **Dr. Peijun Zhang, pez7@pitt.edu**.

RESEARCH TECHNICIAN

Will assist with cell cultures, developing fluorescence/EM labeling probes, fluorescence microscopy studies and other experiments. Qualifications include a Bachelor or Master degree in biological sciences and/or engineering. A background in cell biology, biochemistry, and engineering would all be suitable. Interested candidates can directly send a CV including areas of expertise and interest, publications list, and names and contact information of three references to **Dr. Peijun Zhang, pez7@pitt.edu**.

WEB DEVELOPER/PROGRAMMER

The project includes: (i) building a database to store experimental and modeling data gathered by HIV protein interaction researchers, (ii) building a web server for the tools and data generated by the center, (iii) design of an interactive website that would make these tools and data available to the public, (iv) design of communication means to facilitate collaboration between research teams. Applicant must have experience with contemporary web development techniques, database design, and good project management skills. EDUCATION: 4 Years College - Degree completion not necessary if experience appropriate. SKILLS REQUIRED: Servelet/Java/HTML/SQL/PHP or ASP.net/JSP/CSS. Contact: **Dr. Judith Klein-Seetharaman, jks33@pitt.edu**.

POSTDOCTORAL ASSOCIATE

Highly motivated individual needed to study HIV-1 viral protein and host protein interactions. A Ph.D. degree with strong background in biochemistry, molecular biology, and tissue culture is required. Prior experience in protein expression and purification systems is highly desirable. Please send curriculum vitae and names and contact information of three references to **Dr. Velpandi Ayyavoo, Velpandi@pitt.edu**.

RESEARCH ASSOCIATE – PROTEIN CORE

He/She will utilize molecular biological procedure, including PCR, cloning, protein expression, and purification in bacterial, yeast, insect cell system to support structural characterization of proteins. Expertise in protein purification utilizing FPLC and HPLC and biophysical characterization of proteins are required. Experience in mammalian cell tissue culture work is a plus. Excellent organization and communication skills are essential for multi-faced projects. Applicants should have a Ph. D. or M.S. with equivalent experience. Please send C.V. and names of three references to **Dr. Jinwoo Ahn, jia12@pitt.edu**.

X-RAY FACILITY MANAGER

Seeking an experienced and highly motivated person for managing our macromolecular X-ray crystallography facility and will report directly to the Director (**Joanne I. Yeh, Ph.D.**). The successful candidate will train staff in the use of X-ray crystallographic equipment, software, and robotics. The manager will interface with users on the use of equipment, providing scientific and technical guidance as needed. The facility houses two high intensity FR-E generators, with four detectors including two Saturn 944 CCDs, a RAXIS IV^{++} image plate, and a RAXIS HTC image plate detector. All detectors are equipped with VariMax optics and X-stream 2000 cryogenic systems. An ACTOR robot is also available for automatic phi-mounting and screening of crystals. The facility manager will also coordinate scheduling and allocation of general users time and maintain usage logs, with the guidance of the Director. The manager may also help in data collection at synchrotron beamlines. Experience with handling, cryo-cooling, and manipulation of macromolecular crystals and in structure determination and refinement required. Prior X-ray facilities management experience a plus but not required. Applicants should have a PhD or equivalent experience. Contact: **Dr. Joanne Yeh, jiyeh@pitt.edu**.

TWO POSTDOCTORAL ASSOCIATE POSITIONS – MACROMOLECULAR X-RAY CRYSTALLOGRAPHY

Highly motivated individuals will conduct research as part of an interdisciplinary team; one project is focused on structure-based HIV inhibitor design and drug discovery; the other on structural studies of HIV protein complexes. Candidates should have a Ph.D. with training in crystallography and be experienced in protein crystal structure determination, including protein crystallization, data collection and processing, structure determination, and refinement. Computational experience a plus for inhibitor-based project but not required. Prior experience on AIDS-related structural work is not required; strong crystallographic training is essential. Interested applicants should send their CV and the names of 3 references to **Dr. Joanne Yeh, jiyeh@pitt.edu**.

RESEARCH ASSOCIATE – PROTEIN NMR STRUCTURAL BIOLOGIST

Seeking one highly motivated postdoctoral researcher experienced in macromolecular protein-protein complex structure determination by state-of-the-art solution NMR spectroscopy. Dept of Structural Biology is equipped with a total of six state-of-the-art spectrometers including 900, 800, 700 and three 600 MHz spectrometers, fully dedicated for biological NMR and equipped with multi-resonance z-axis gradient cryoprobes with deuterium decoupling capability. Interested candidates can directly send a CV and names and contact information of three references to **Dr. In-Ja L. Byeon, ilb6@pitt.edu**.

ASSISTANT PROFESSOR

Systematic Invertabrate Zoology

We invite applications for a tenure-track assistant professor position in Systematic Invertebrate Zoology to begin August 2008. Candidates must have a Ph.D., postdoctoral research experience and a research plan that integrates modern molecular approaches to study the systematics, biogeography, and evolution of freshwater invertebrates. Candidates working on systematics of any group of freshwater invertebrates will be considered. Candidates will be expected to curate one of the freshwater invertebrate collections of Biological Sciences (e.g., Malacology, Decapods, etc.). Candidates must provide evidence of curatorial experience and/or other relevant abilities. Applicants are advised to view a more detailed job description at **www.as.ua.edu/biology** prior to submitting their application package. Successful candidates will have demonstrated excellence in research and will be expected to attract extramural funding. Candidates must be committed to excellence in teaching and training of undergraduate and graduate students. Opportunities for interactions exist through the Center for Freshwater Studies, Coalition for BioMolecular Products, and Alabama Museum of Natural History.

To apply, mail hardcopies of *curriculum vitae*, statements regarding research goals, teaching philosophy and interests, evidence of curatorial experience, and copies of significant publications, and have three reference letters sent to: **Invertebrate Systematist Search Committee, Department of Biological Sciences, Box 870344, The University of Alabama, Tuscaloosa, AL 35487**. Review of applications will begin January 2, 2008, and will continue until the position is filled.

The University of Alabama is an Affirmative Action/Equal Opportunity Employer. Applications from women and minorities are encouraged.

Crimson is
THE UNIVERSITY OF ALABAMA

UNIVERSITY of VIRGINIA
HEALTH SYSTEM

HUMAN GENETICS/GENOMICS

The Center for Public Health Genomics at the University of Virginia invites applications for positions at the Assistant to Full Professor level with research interests in human molecular genetics/genomics, particularly in the genetic basis of complex human disease.

Rank of appointment is dependent upon prior experience and research accomplishment. These faculty positions are being jointly recruited in the new multidisciplinary Center for Public Health Genomics with primary academic appointment in an existing Department within the University of Virginia School of Medicine. Candidates who complement existing research strengths in cancer, cardiovascular disease, obesity, autoimmunity, and neurodegenerative diseases are particularly encouraged to apply.

Candidates will have a Ph.D. and/or MD degree and exceptional potential for establishing both collaborative and independent research. Newly renovated, high-quality laboratory space in an exceptional computing support environment will be provided. We strongly encourage applications from women and minorities.

Positions are available immediately. Applications should include curriculum vitae, email address, a brief statement of proposed research program, and names and addresses of three references. Please send materials (electronically) to:

Search Committee
Center for Public Health Genomics
University of Virginia School of Medicine, P.O. Box 800717
Charlottesville, VA 22908-0717

cphg-search@virginia.edu

The University of Virginia is an Equal Opportunity/ Affirmative Action Employer.

REGINALD A. DALY POSTDOCTORAL FELLOWSHIP
HARVARD UNIVERSITY
DEPARTMENT OF EARTH AND PLANETARY SCIENCES

The Department of Earth and Planetary Sciences at Harvard University invites applicants for the Reginald A. Daly Postdoctoral Research Fellowship.

The Department seeks outstanding candidates in the broad field of Earth and Planetary Sciences. **We encourage applications of candidates pursuing field observations, lab-based science, and theory, and interested in geology, geochemistry, ocean, atmosphere and climate dynamics and chemistry, seismology, geophysics, planetary sciences, and other related fields.** These honorific postdoctoral fellowships are awarded for a one-year period, with an anticipated extension for a second year. Daly fellows carry out independent research, yet are encouraged to interact with one or more research groups in the department. Applicants are welcome to contact members of the department before applying. Applications should include a curriculum vitae, names and affiliation of three referees, a one page statement of the applicant's doctoral research, and a one to two page postdoctoral research proposal. Applications are due **January 15, 2008**. Applicants are responsible for contacting the referees to have their letters arrive directly at the address below by the **January 15, 2008** deadline. Send applications by email to: **Daly Postdoctoral Search Committee, dalypostdoc@eps.harvard.edu, Department of Earth and Planetary Sciences, Harvard University, 20 Oxford Street, Cambridge, MA 02138.**

The annual salary is $52,000 with additional funds of $15,000 available for research support over a two-year period. Applicants should have a recent Ph.D. or should be 2008 degree candidates. Completion of the Ph.D. is required by the time of the appointment. For more information about the department and the Daly postdoctoral program, please visit **http://www.eps.harvard.edu/daly.php**.

We particularly encourage applications from women and minorities. Harvard University is an Affirmative Action/ Equal Opportunity Employer.

YALE UNIVERSITY
School of Forestry and Environmental Studies
Faculty Position
Terrestrial Ecosystem Ecology

Yale University's School of Forestry and Environmental Studies seeks to hire a tenure track or tenured faculty member in Terrestrial Ecosystem Ecology. Senior candidates will have developed a highly regarded field-oriented research program, have a demonstrated capacity for interdisciplinary research, and possess a very strong record of publication. Junior candidates will have shown the potential for developing such a research program, with a record of publishing, and a demonstrated enthusiasm for interdisciplinary and applied research. He or she will have broad knowledge of terrestrial ecosystem ecology. Subject areas of interest are broad, but examples are: plant-water-soil dynamics, soil biogeochemistry, plant diversity-soil interactions and soil ecology/microbial ecology. Candidates with strong field skills and demonstrated experimental research that scale across systems and/or is comparative are preferred. Candidates should be prepared to teach graduate-level courses on soils and ecosystem ecology, as well as advanced seminars on more specialized topics.

Applicants should send by **15 November 2007** their curriculum vitae, a statement of their research and teaching interests, a list of three references, and representative examples of their publications to:

Terrestrial Ecosystem Ecology Search Committee
c/o Assistant Dean Jane Coppock
Yale School of Forestry and Environmental Studies
205 Prospect Street, New Haven, CT 06511
USA

Or by email to: **jane.coppock@yale.edu**

Additional information on this position may be obtained by contacting **Professor Mark Ashton, 205 Prospect Street, New Haven, CT 06511, USA, phone: (203) 432-9835, email: mark.ashton@yale.edu**.

Yale University is an Affirmative Action /Equal Opportunity Employer. Members of minority groups and women are encouraged to apply.

SCIENTIST IN REPRODUCTIVE BIOLOGY
WOMEN'S HEALTH AND INFANT DEVELOPMENT RESEARCH CENTER
EASTERN VIRGINIA MEDICAL SCHOOL

The newly established Women's Health and Infant Development Research Center at Eastern Virginia Medical School (EVMS) is seeking an investigator to fill a tenure-track faculty position in the area of reproductive/perinatal endocrinology. EVMS is making a major commitment to strengthen, expand and integrate the academic enterprise in basic and clinical research. The Women's Health and Infant Development Research Center spans across and is to be comprised of investigators from several departments and the new recruit will hold a primary academic appointment within the Department of Obstetrics and Gynecology. The candidate is expected to have a Ph.D. and/or M.D. and postdoctoral training in the reproductive sciences, a strong background in molecular biology, U.S. citizenship, and active NIH RO1-type research grant funding. Opportunity exists at EVMS for collaboration in non-human primate pregnancy and reproduction research. Applicants should submit a letter of interest, an abbreviated NIH biosketch and full CV to: Eastern Virginia Medical School, Women's Health and Infant Development Research Center, Department of Obstetrics and Gynecology, Attention: Kerry Jones, 601 Colley Avenue, Suite 233, Norfolk, VA 23507, email: joneska@evms.edu.

EVMS
Eastern Virginia Medical School

Johns Hopkins Medical Institutions
Tenure-Track Positions

Influenza and Respiratory Virus Translational Research

Human Immunology, Vaccinology, Pharmacology

The Division of Infectious Diseases of the Johns Hopkins School of Medicine is recruiting 1-2 faculty at the Assistant or Associate Professor level to contribute to an emerging institutional Respiratory Viruses Program. Our focus is on persons with proven capabilities to conduct independent research on respiratory infections, especially investigations that contribute to the prevention or treatment of influenza in humans. This recruitment contributes to expanding programs in influenza virology, structural biology, and vaccine testing. Emphasis will be given to researchers with complementary research such as in molecular biology of viral replication, host virus interactions, and quantitative analysis of viral dynamics.

Candidates must have earned an MD and/or PhD degree and have a record of acquiring research funding and producing outstanding scholarship. Salary and resources will match experience.

Candidates should provide a curriculum vitae, a one-page statement of career interest, and 3 professional references to: **Dr. David Thomas, Chief Infectious Diseases, Johns Hopkins School of Medicine, Suite 437 1830 Monument Street, Baltimore, Maryland 21205** or by email care of Nadia Hay **nhay@jhmi.edu**. Application review will begin in Fall 2007.

Johns Hopkins is an Equal Opportunity Employer.

UNIVERSITY OF CALGARY

TENURE-TRACK FACULTY POSITION
TOXICOLOGY/CLINICAL TOXICOLOGY
FACULTY OF VETERINARY MEDICINE

Applications are invited for a tenure-track faculty position in toxicology or clinical toxicology in the University of Calgary **Faculty of Veterinary Medicine** (UCVM).

The successful candidate will be expected to contribute to teaching in the undergraduate DVM and graduate programs and to develop an externally funded research program. While excellent individuals in any area of toxicology or clinical toxicology will be considered, UCVM is particularly interested in individuals with expertise in environmental toxicology, food safety, oil/gas field-related toxicology, or other areas relevant to Alberta.

Teaching duties will include teaching of toxicology/clinical toxicology in DVM program and the graduate program, supervision of graduate students, and other relevant educational activities.

Qualifications include a PhD in toxicology in a related field and/or a DVM degree with advanced training in toxicology/clinical toxicology. The ability to develop or maintain an externally funded research program and strong communication and educational skills are required. The appointment rank (assistant professor or higher) and salary will be commensurate with experience. The appointment will be in a department that aligns with the research and teaching interests of the successful candidate.

UCVM will be Canada's fifth accredited veterinary college and will accept its first class of 30 students in September 2008. The UCVM program will deliver a comprehensive general veterinary education and provide enhanced educational opportunities in production animal health, equine health, ecosystem and public health, and investigative medicine. The four-year curriculum combines a clinical presentations model with discipline-based courses and extensive on-campus and off-campus practical experiences during the first three years of the program. UCVM will use a distributed veterinary learning community including public and private practice partners to deliver its fourth year practicum program. UCVM faculty will work across disciplines and departments, fostering collaborative research, teaching and service. Descriptions of the Faculty and its departments can be found on the UCVM website (www.vet.ucalgary.ca).

The University of Calgary is a comprehensive, research-intensive university. Calgary is a vibrant, multicultural city of 1,000,000 near the Rocky Mountains, Banff National Park and Lake Louise. Alberta has a large agri-food and oil industry economic base.

Review of applications will begin **December 1, 2007** and continue until the position is filled. Interested individuals should submit a curriculum vitae, a statement of teaching and research interests, and the names of three referees to:

Dr. Alastair Cribb, Dean
Faculty of Veterinary Medicine
3330 Hospital Drive N.W.
Calgary, AB T2N 4N1 Canada
e-mail: vetdean@ucalgary.ca

Those applicants wishing more information are encouraged to visit the website or contact Dr. Alastair Cribb (acribb@ucalgary.ca; 403-220-3790).

In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. The University of Calgary respects, appreciates, and encourages diversity.

www.ucalgary.ca

POSITIONS OPEN

TENURE-TRACK FACULTY POSITION
Molecular Physiology

Furman University invites applications for a tenure-track position at the **ASSISTANT PROFESSOR** level beginning August 2008. The successful candidate will be a **MAMMALIAN PHYSIOLOGIST** whose research includes work at the molecular level and who will utilize our new vivarium facility. Teaching responsibilities will include human physiology, animal physiology, introductory biology, and upper-level course(s) in an area of specialty. Candidates must have the Ph.D., evidence of interest and excellence in teaching, and a commitment to involving undergraduate students in an active research program. Furman is a selective, independent liberal arts school of 2,600 students, with a strong emphasis on engaged learning and undergraduate research. Start-up funds will be available. For further information, see **website: http://www.furman.edu/academics/biology/job/.** Submit curriculum vitae, statement of teaching credentials and philosophy, description of research interests, unofficial transcripts, and three letters of recommendation to: **Dr. Dennis Haney, Chair, Molecular Physiologist Search Committee, Department of Biology, Furman University, 3300 Poinsett Highway, Greenville, SC 29613** or **e-mail: dennis.haney@furman.edu.** Review of applications will begin November 12, 2007, continuing until the position is filled. *The University and the Department have a strong commitment to achieving diversity among faculty and staff. We strongly encourage women and people from diverse racial, ethnic, and cultural backgrounds to apply. Equal Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSOR. Auburn University **(website: http://www.auburn.edu/),** Department of Entomology and Plant Pathology, **(website: http://www.ag.auburn.edu/enpl),** is accepting applications and nominations for the position of Assistant Professor in Applied Insect Ecology. For a detailed position announcement, including application instructions and requirements, please visit **website: http://www.ag.auburn.edu** and select Employment on the webpage. Inquiries about the position may be directed to: **Dr. William J. Moar, Search Committee Chair, Department of Entomology and Plant Pathology, Auburn University, AL 36849; e-mail: moarwil@auburn.edu.** Active review of applications will begin on January 8, 2008, and continue until the search is filled. Only complete application materials will be considered. To ensure full consideration for the position, applicants are encouraged to submit all materials by January 7, 2008. Expected position start date is August 16, 2008. *Minorities and women are encouraged to apply. Auburn University is an Affirmative Action/Equal Opportunity Employer.*

PLANT PHYSIOLOGIST

University of Wisconsin, Eau Claire, a comprehensive liberal arts-based university and Center of Excellence in Undergraduate Research, seeks a tenure-track **ASSISTANT PROFESSOR** beginning August 19, 2008, to serve in a highly active and collegial Department of Biology. Ph.D., demonstrated ability to teach at post-secondary level, and a commitment to develop an active research program involving undergraduates required. Responsibilities include teaching plant physiology, organismal form and function, and additional courses that are currently offered or would complement the curriculum. Detailed description and application information at **website: http://www.uwec.edu/biology.** Priority deadline December 3, 2007. Send inquiry to: **Plant Physiologist Search, Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, WI 54702-4004. E-mail: bogstafg@uwec.edu. Telephone: 715-836-4166. Fax: 715-836-5089.** *Equal Opportunity/Affirmative Action Employer.*

STEM CELL INVESTIGATORS
Biology, Medicine, Engineering, Physical Sciences, or Any Other Field
Fast Track to Independence

The UCSD Stem Cell Program at the University of California, San Diego in La Jolla, California, is seeking to recruit the world's most outstanding early-career Research Scientists or Physician/Scientists in disciplines related to stem cell science and medicine upon completion of their Ph.D. or M.D. degrees. Successful candidates will conduct research the first year in an existing La Jolla laboratory or academic group, and then be given space and support for up to a total of four years to pursue independent research. The successful applicants will have appointments in one or more home departments at UCSD, and will be expected to contribute to the vibrant, collaborative, cross-disciplinary life of the UCSD Stem Cell Program.

The Program particularly seeks investigators who will establish innovative approaches and have expertise in more than one discipline related to stem cell research.

Applicants whose research bears on understanding and treatment of Alzheimer's disease are invited to apply for the position of **KAEHR STEM CELL YOUNG INVESTIGATOR.** Applicants in other fields are sought for other open positions.

Scholars will be provided funds for salary, supplies, expenses, travel, and laboratory support, information technology and administrative support, and laboratory space for four years (non-renewable). Salary will be commensurate with experience, following the University of California salary scales. An attractive benefits package is included. Title, rank, and salary will depend on a candidate's skills, qualifications, and experience. Preference is given to early-career scientists, but applicants with more developed careers will also be considered. Recipients of these positions must hold the Ph.D. or M.D. and have the potential for independent research careers.

For more information on our program and to apply, please go to **website: http://stemcells.ucsd.edu/.**

You may also send a nomination with curriculum vitae, research experience and interests, and the names of three references, to **e-mail: jbraswell@ucsd.edu.**

Faculty may nominate promising scholars, and self-nominations are also welcome. The UCSD Stem Cell Program will invite promising scholars to complete a full application. Review will begin November 9, 2007, and will continue until all positions are filled. *Equal Opportunity/Affirmative Action Employer.*

PLANT BIOLOGIST. Position #103390. Middle Tennessee State University, with over 23,000 students enrolled, invites applications for a tenure-track Plant Biologist position with expertise in plant ecology or systematics. Ph.D. required, postdoctoral experience preferred, rank open. The successful candidate will be expected to direct an extramurally funded research program involving graduate and undergraduate students, and take part in proposed Ph.D. programs. The Department of Biology is a scientifically diverse research active Department offering B.S. and M.S. degrees. Members are committed to research, quality classroom teaching, as well as professional service. Individuals with an interest in cedar glades or other regionally significant plant communities are particularly encouraged to apply. Teaching responsibilities may include introductory biology and an upper-division course in area of specialty. Application materials must be filed online at **website: http://www.mtsujobs.mtsu.edu.** Complete application materials for preliminary review to include: letter of interest, employment application, copies of transcripts, and curriculum vitae. Review of applications begins November 12, 2008. For additional information contact **Committee Chair, Dr. A. Bruce Cahoon, e-mail: acahoon@mtsu.edu.** More information about Middle Tennessee State University can be found at **website: http://www.mtsu.edu/.**

Equal Opportunity/Affirmative Action/ADA Employer.

POSTDOCTORAL POSITIONS available until filled for highly motivated individuals with Ph.D. in molecular biology/ microbiology/ computational structural biology/ biochemistry/ chemical engineering, or related disciplines. Successful candidates will play a key role in a multidisciplinary effort to design, synthesize, screen, and characterize novel biomacromolecules or metabolic pathways and to develop cutting-edge technologies for biomolecular or genome engineering. Individuals with a productive track record and skills in molecular cloning, protein expression, enzyme assays, high throughput screen, microbial genomics, or protein design and structural characterization are encouraged to apply. Send curriculum vitae with names of three references to **Professor Jingdong Tian, Biomedical Engineering and Genome Sciences, Duke University** by **e-mail: jtian@duke.edu.**

THREE ASSISTANT PROFESSORS of MARINE SCIENCE

Coastal Carolina University (CCU) invites applications for the following tenure-track Assistant Professor positions in the Department of Marine Science beginning fall 2008.

(1) Assistant Professor of Marine Science **(MARINE SYSTEMS MODELER),** one position. The University seeks a highly motivated individual with a commitment to undergraduate and graduate education and an established record of research accomplishments. Preferences will be given to candidates with expertise in marine ecosystem/ecological models. Strong candidates will also be considered in other modeling areas of research, including physical flow/transport models, marine/coastal resources economics, and/or geographic information systems applications.

(2) Assistant Professor of Marine Science **(COASTAL MARINE GEOSCIENTISTS),** two positions. The University seeks highly motivated individuals with a commitment to undergraduate and graduate education and an established record of research accomplishments. Candidates will be expected to have a coastal marine geoscience focus. Applied hydrogeology is preferred for one of the positions while other areas of preference are coastal Quaternary studies, coastal sedimentary environments or processes, or estuarine/nearshore dynamics.

The Department of Marine Science is committed to a collaborative, interdisciplinary philosophy of education and research. The successful candidate will be expected to teach introductory and upper-division courses in the undergraduate Marine Science Program, as well as graduate courses in the Coastal Marine and Wetlands Studies Program. The candidate will be expected to develop a successful externally funded research program involving both undergraduate and graduate students. Applicants must have a Ph.D. in marine science or a related field.

Coastal Carolina University is a public mid-sized, comprehensive liberal arts-oriented institution. Coastal Carolina University is located in Conway, South Carolina, just nine miles from the Atlantic coastal resort Myrtle Beach, one of the fastest-growing metropolitan areas in the nation. It has an enrollment of 8,400 students and is expected to have continued growth for the next several years. Coastal Carolina University is a part of the South Carolina system of public education and has close ties with its founders, the Horry County Higher Education Commission.

Interested candidates should submit a letter of application, curriculum vitae, statement of teaching and research interest, and the contact information for at least three professional references, electronically at **website: http://jobs.coastal.edu.** Review of applications will begin November 1, 2007, and continue until the position is filled. For further information about CCU and marine science visit **website: http://kingfish.coastal.edu/marine.** *Coastal Carolina University is an Equal Opportunity/Affirmative Action Employer.*

Faculty Openings, Department of Biology
Brigham Young University

The review process will begin **November 1, 2007** for all positions and continue until the positions are filled. Faculty application forms can be found at: **https://yjobs.byu.edu**. Additional department and college information is available at website: **http://biology.byu.edu/home**.

Bioinformatics/Computational Biology

The Department of Biology at Brigham Young University seeks to fill two continuing faculty status track positions in the areas of bioinformatics and/or computational biology. We seek exceptional individuals with a PhD and postdoctoral experience relevant to bioinformatics, genomics, and/or computational biology, including degrees in areas of biology, computer science, mathematics, and/or statistics. The successful candidates are expected to develop an externally funded research program and teach courses in Bioinformatics and the biology core. The department offers competitive start-up packages and reduced teaching loads for new faculty.

Interested persons should send a CV, statements of teaching and research interests, and a completed BYU faculty application form to: **Dr. Keith Crandall, 401 Widtsoe Building, Department of Biology, BYU, Provo, UT 84602**, electronic applications preferred to **bio@byu.edu**. For further information on the bioinformatics program at BYU see **http://bioinformatics.byu.edu/**.

Ecology/Evolution

The Department of Biology is seeking outstanding colleagues to join an active and interdisciplinary faculty with strengths in evolutionary and organismal biology, ecology, and biological science education. We seek qualified applicants to fill one or more continuing faculty status track positions in any area of evolutionary biology or population/evolutionary ecology. The successful candidate will hold a PhD, have post-doctoral experience, and is expected to maintain an externally funded research program involving both undergraduate and graduate students. Excellence in teaching is expected and college-level teaching experience is preferred.

Teaching responsibilities will vary with the candidate's background and will include a contribution to our undergraduate curriculum (e.g., general biology, ecology, evolution, conservation biology, or plant biology) as well as a graduate course in the candidate's area of expertise. The department offers competitive start-up packages and reduced teaching loads for new faculty.

Interested persons should send a CV, statements of teaching and research interests, and a completed BYU faculty application form to: **Dr. Jerry Johnson, Ecology/Evolutionary Biology Search Committee Chair, 401 WIDB, Department of Biology, BYU, Provo, UT 84602**, electronic applications preferred to **bio@byu.edu**.

Biologist

The Department of Biology is offering a continuing faculty status track position (open rank) to begin in the fall of 2008 (negotiable). Outstanding candidates with expertise in any area of biology will be considered, although preference will be given to those applicants who integrate with several of the department research foci in evolution and ecology, bioinformatics, conservation biology, botany, or science education. Candidates must have a Ph.D. in biology or a related discipline and postdoctoral experience and will be expected to maintain an externally funded research program involving both undergraduate and graduate students. Excellence in teaching is expected with responsibilities varying with the candidate's background, but including a contribution to the undergraduate curriculum (e.g., general biology, biological science education, ecology, evolution, conservation biology, or plant biology) as well as a graduate course in the candidate's area of expertise. The department offers competitive start-up packages and reduced teaching loads for new faculty.

Interested persons should send a CV, statements of teaching and research interests, and a completed BYU faculty application form to: **Dr. Mark Belk, Biology Search Committee Chair, Brigham Young University, Provo, UT 84602**, electronic applications preferred to **bio@byu.edu**.

BYU, an Equal Opportunity Employer, is sponsored by The Church of Jesus Christ of Latter-day Saints and requires observance of Church standards. Preference is given to members in good standing of the sponsoring Church.

UT SOUTHWESTERN MEDICAL CENTER

Translational Oncology Research Opportunities at UT SOUTHWESTERN MEDICAL CENTER at DALLAS

The Simmons Cancer Center and Department of Internal Medicine Division of Hematology Oncology at the University of Texas (UT) Southwestern Medical Center invite applications for tenure track faculty appointments at the level of Assistant Professor. Successful candidates will have outstanding start-up support and protected time to develop a disease focused research program. Applicants must have an M.D. or M.D., Ph.D., postdoctoral experience, and demonstrate the ability to develop an independent research program as well as an interest in translational oncology.

Simmons Cancer Center translational research faculty will be housed in state-of-the-art laboratories in the new T. Boone Pickens Research Building and will be appointed in the Department of Internal Medicine. Faculty will be encouraged to participate in cancer center research programs led by renowned cancer investigators including **Drs. Luis Parada, Jerry Shay, Melanie Cobb, Michael White, Steven McKnight, David Chen,** and **John Minna**. Joint appointments in a basic science department are possible and faculty will be eligible to participate in the Cancer Biology Track of the UT Southwestern Graduate School of Biomedical Sciences. The presence of clinical programs in cancer research, treatment, and prevention offer unique opportunities for creative collaboration, and a tissue procurement and tumor bank provide investigators access to clinical specimens.

Please send curriculum vitae, a summary of current and proposed research programs, and arrange for three letters of recommendation to be sent to:

James K. V. Willson, M.D.
Director, Simmons Cancer Center
or Joan Schiller, M.D., Chief
Hematology Oncology
UT Southwestern Medical Center
5323 Harry Hines Blvd.
Dallas, Texas 75390-8590

E-mail: **james.willson@utsouthwestern.edu** or **joan.schiller@utsouthwestern.edu**.

UT Southwestern is an Equal Opportunity/ Affirmative Action Employer.

POSITIONS OPEN

GORDON A. and MARY CAIN CHAIR in CHEMICAL ENGINEERING #TWO and PROFESSOR
Department of Chemical Engineering

The Gordon A. and Mary Cain Department of Chemical Engineering is seeking highly qualified candidates for a second established Chair supported by the Cain Endowment. Funding through both the University and the Endowment will be available for research, staff, and graduate student support. Louisiana State University at Baton Rouge has an increasing enrollment of 30,000 students. Louisiana State University (LSU) holds the Carnegie Foundation's designation as a Doctorate-granting University, with very high research activity. The Chemical Engineering Department offers undergraduate, M.S., and Ph.D. degrees. Research in the Department focuses on a number of key areas such as: advanced computation, environment, materials, energy, biochemicals and systems, with strong commitment to collaboration between these areas towards a truly multidisciplinary research effort. Research in the Department is funded from external grants and contracts from several federal agencies. The LSU Department of Chemical Engineering has consistently been among the top departments in the nation in this measure of research activity **(website: http://www.che.lsu.edu).** More information about LSU and the Baton Rouge area can be obtained by visiting **website: http://www.lsu.edu.**

Required qualifications: Ph.D. or equivalent degree in chemical engineering or a closely related field; proven track record in scholarly activity, teaching, professionalism, and service or equivalent industrial experience. Responsibilities: teaches both graduate and undergraduate students; commits to excellence in research. Preference will be given to candidates or nominees with proven track record and activity in the area of renewable energy/resources and clean energy; however, candidates with strong background in areas such as materials, bioprocessing, computational and environmental engineering will also be considered. *An offer of employment is contingent on a satisfactory pre-employment background check.* Application deadline is January 18, 2008, or until a candidate is selected. Submit nominations, request for information, or a letter of application and resume (including e-mail address) to:

Head, Gordon A. and Mary Cain Chair Search Committee
Gordon A. and Mary Cain
Department of Chemical Engineering
Louisiana State University
Reference: #028449
Baton Rouge, LA 70803-7303
Telephone: 225-578-1426

LSU is an Equal Opportunity/Equal Access Employer.

POSTDOCTORAL RESEARCH ASSOCIATE POSITION is available immediately for a highly motivated individual interested in cellular and molecular mechanisms of immune system activation and autoimmunity. Qualifications: strong background in molecular biology, immunology, biochemistry, and the ability to work independently and cooperatively with other members in the Laboratory. Experience with immunological analysis of rodent models is desired. Ph.D. or M.D./D.V.M., or equivalent required. Review of applications will begin on November 15, 2007, and will continue until the position is filled or closed. Send application letter, statement of interest and relevant work experience, curriculum vitae, and three references by mail or e-mail to: **Jay Reddy, M.V.Sc., Ph.D., Associate Professor, Department of Veterinary and Biomedical Sciences, University of Nebraska-Lincoln, 202 VBS, Lincoln, NE 68583-0905; e-mail: nreddy2@unl.edu.** *University of Nebraska-Lincoln is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity; and assures reasonable accommodation under the Americans with Disabilities Act.*

POSITIONS OPEN

VCU

TENURE-TRACK FACULTY POSITION DIRECTOR of FUNCTIONAL LIPIDOMICS/METABOLOMICS INITIATIVE
Virginia Commonwealth University
School of Medicine

Virginia Commonwealth University (VCU) School of Medicine is expanding an initiative in functional lipidomics/metabolomics and invites applications for a tenure-track faculty position to spearhead this initiative. Candidates should have a research program with a record of sustained productivity and current extramural funding. Substantial resources are available to support recruitment of an outstanding investigator at the rank of **ASSISTANT, ASSOCIATE, or FULL PROFESSOR,** based upon qualifications and experience. VCU has a very active and expanding critical mass of investigators whose research is focused on metabolism and signaling of bioactive lipids in cancer, inflammation, atherosclerosis, heart and lung disorders, cholesterol and bile acid metabolism in liver disorders, and insulin resistance and fatty liver disease. These programs have a history of strong and successful research and training programs. However, this search is not necessarily focused on existing areas of strength and invites outstanding applications covering any aspect of lipidomics or metabolomics. More information about the School of Medicine and Departments, and this open position can be found at **websites: http://www.vcu.edu/biochem/department/pos.shtml** and **http://www.pubinfo.vcu.edu/facjobs/facjob.asp?Item=2290.** Applicants should submit by e-mail curriculum vitae, names and e-mail addresses of three references, and a summary of research and teaching interests to: **Dr. Robert F. Diegelmann (e-mail: rdiegelm@vcu.edu), Department of Biochemistry, Virginia Commonwealth University School of Medicine.** *Virginia Commonwealth University is an Equal Opportunity/Affirmative Action Employer. Women, persons with disabilities, and minorities are encouraged to apply.*

ASSISTANT, ASSOCIATE, or FULL PROFESSOR/CURATOR
Evolution and Systematics of Fungi
University of Michigan

The Department of Ecology and Evolutionary Biology and the University Herbarium seek applications for a tenured or tenure-track faculty position in the evolution and systematics of fungi. The position will have a University-year appointment. Depending on rank, the successful candidate may be appointed to the Wehmeyer Chair in Fungal Taxonomy. We seek outstanding individuals whose primary research interests are in aspects of fungal evolutionary biology such as molecular evolution and systematics, evolution of adaptation, or evolution of development. We are especially interested in individuals who can place evolutionary processes in ecological contexts through collaborations with colleagues. Teaching may include a course in fungal evolution or diversity, and contributions to core courses in introductory biology, evolution, or genetics. The candidate will also provide scholarly leadership in the use of the Herbarium's outstanding research collection. For additional information, see **websites: http://www.eeb.lsa.umich.edu** and **www.herbarium.lsa.umich.edu.** To apply, send curriculum vitae, statements of current and future research plans and of teaching philosophy and experience, evidence of teaching excellence, and copies of publications, as well as arrange to have three reference letters mailed to: **Chair, Fungal Evolution and Systematics Search Committee, Department of Ecology and Evolutionary Biology, The University of Michigan, 830 N. University, 2019S Kraus, Ann Arbor, MI 48109-1048.**

Review of applications will begin on December 1, 2007. *The University of Michigan is an Equal Opportunity, Affirmative Action Employer. Women and minorities are encouraged to apply. The University is supportive of the needs of dual-career couples.*

POSITIONS OPEN

ASSISTANT PROFESSORS of BIOLOGY
(Four Positions)

The Department of Biology has initiated a new curriculum in response to rapid institutional growth. We plan to fill four full-time, tenure-track positions at the Assistant Professor level beginning August 2008. The Ph.D. is required.

(1) **PLANT MOLECULAR BIOLOGIST:** Interests include using molecular methods to investigate basic plant biology, plant/microbe interactions, or genetic modifications for agricultural and pharmaceutical purposes.

(2) **MOLECULAR CELL BIOLOGIST or MOLECULAR MICROBIOLOGIST:** Interests include using molecular and computational methods to investigate areas of cell or microbiology including regulation of gene expression or comparative analyses of bacterial genomes and proteomes. Successful candidates for the two molecular positions will be responsible for teaching either cell biology, genetics or microbiology and an advanced course in the applicant's area of specialization.

(3) **VERTEBRATE BIOLOGIST:** Candidates from a broad range of research specialties including conservation, evolution, functional anatomy or physiology are encouraged. Our Program and region offer excellent opportunities for **ICHTHYOLOGISTS** or **HERPETOLOGISTS.** Teaching responsibilities include comparative vertebrate anatomy and an advanced or graduate course in the candidate's area.

(4) **ANIMAL PHYSIOLOGIST:** Broadly trained candidates from research specializations in ecological physiology, endocrinology, sensory physiology, neurophysiology, or biomechanics are encouraged. Teaching responsibilities include an upper-level course in animal physiology and an advanced or graduate course in the candidate's area.

For all four positions, successful candidates will be expected to emerge as exemplars of teaching, to contribute to introductory courses, and to develop potentially fundable research programs involving undergraduates, and, where applicable, graduate students. The Biology Department has 400 undergraduate majors, 18 graduate students, 15 full-time faculty, and five adjunct faculty. Undergraduate students earn a B.S. degree in biology. The Department also participates in an interdisciplinary M.S. in coastal marine and wetland studies, and offers courses for graduate students in the Masters of Arts in Teaching Program in the Spadoni College of Education. For departmental information go to **website: http://www.coastal.edu/biology.** Coastal Carolina University is a public mid-sized, comprehensive liberal arts-oriented institution. Coastal Carolina University is located in Conway, South Carolina, just nine miles from the Atlantic coastal resort Myrtle Beach, one of the fastest-growing metropolitan areas in the nation. It has an enrollment of 8,400 students and is expected to have continued growth for the next several years. Coastal Carolina University is a part of the South Carolina system of public education and has close ties with its founders, the Horry County Higher Education Commission. Interested candidates should submit a letter of application, curriculum vitae, statement of teaching and research goals, and the contact information for at least three professional references, electronically at **website: http://jobs.coastal.edu.** Review of applications will begin November 1, 2007, and continue until the position is filled. *Coastal Carolina University is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITIONS are open to study biochemical mechanism of ubiquitylation in regulating genomic integrity and tumorigenesis. Candidates with strong background in tumor mouse model, and genomic integrity are encouraged to send curriculum vitae and three references to: **Dr. Yong Wan, Ph.D., University of Pittsburgh Cancer Institute, Hillman Cancer Center, 5117 Centre Avenue, Room 2.6C, Pittsburgh, PA 15213. E-mail: yow4@pitt.edu.** Laboratory **website: http://www.cbp.pitt.edu/faculty/yong_wan.** *The University of Pittsburgh is an Affirmative Action, Equal Opportunity Institution.*

POSITIONS OPEN

ASSISTANT/ASSOCIATE PROFESSOR
Biological Sciences, Multiple Positions
Department of Biological Sciences
St. John's College of Liberal Arts
Queens Campus

St John's University's Department of Biological Sciences invites applicants for two full-time, tenure-track positions at the rank of **ASSISTANT/ASSOCIATE PROFESSOR in BIOLOGICAL SCIENCES** for its Queens campus, starting September 1, 2008. Applicants must possess a Ph.D. and have at least two years of postdoctoral experience, with a strong record of scholarship. Successful candidates will be expected to develop an upper level or graduate course in their specialty, mentor M.S./Ph.D. students, and develop an extramurally funded research program.

One position is for an **ENDOCRINOLOGIST or NEUROBIOLOGIST** and the second one is for a **MICROBIOLOGIST.**

All candidates will be expected to interact extensively with colleagues in a cell and molecular biology-oriented department. We will entertain applications from exceptional candidates in other areas of biology as well.

We offer competitive compensation, excellent benefits, and talented professional colleagues. For consideration, please send an application portfolio containing the curriculum vitae, statement of teaching philosophy, description of research interests and future plans, and three references should be submitted to:

Dr. Jay A. Zimmerman, Ph.D., Chair
Department of Biological Sciences
St. John's University
8000 Utopia Parkway
Queens, NY 11439

E-mail submission preferred, **e-mail: zimmermj@stjohns.edu.**

St. John's University is one of the nation's largest Catholic universities with about 20,000 students and five campuses, four in the New York metropolitan area. See **website: http://www.stjohns.edu.**

St. John's is an Equal Opportunity Employer and encourages applications from women and minorities.

LABORATORY MEDICINE PHYSICIAN-SCIENTIST, MICROBIOLOGY. The Department of Laboratory Medicine at the Yale School of Medicine is actively seeking Physician-Scientist candidates for a new **ASSISTANT/ASSOCIATE PROFESSOR** position in the Microbiology/Virology Section to start July 2008. The successful candidate would be expected to spend approximately 75 percent time in an independent program of basic and/or translational bench research with accompanying 25 percent clinical and teaching responsibilities in the Microbiology and Virology Laboratories. The candidate should be Board-certified (or eligible) in clinical pathology, medical microbiology or infectious disease, with experience in clinical microbiology. The specific area of investigation is open, and could involve host-pathogen interactions, microbial pathogenesis, virology, immunology, or any other relevant field that would interact well with existing institution-wide programs. More information on the Department may be found at **website: http://info.med.yale.edu/labmed.** Please send curriculum vitae and names of three references by January 31, 2008, to the: **Chair of the Search Committee: Paula Kavathas, Ph.D., Department of Laboratory Medicine, 333 Cedar Street, P.O. Box 208035, New Haven, CT 06520-8035. E-mail: microfacultysearch@lab.med.yale.edu; fax: 203-688-4111.** *Yale University is an Affirmative Action/Equal Opportunity Employer. Women and members of minority groups are encouraged to apply.*

POSTDOCTORAL POSITION, University of Montana. MOLECULAR BIOCHEMIST to study transcription-induced hypermutation in human cell lines. Expertise in genetic engineering and cell culture techniques required. Available January 2008. Send curriculum vitae to **e-mail: barbara.wright@mso.umt.edu.** *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

FACULTY POSITION
Biology Department
Boston College

We invite applications for a tenure-track faculty position in the Boston College Biology Department. This search is open to candidates at the level of **ASSISTANT, ASSOCIATE, or FULL PROFESSOR.** The University provides extremely competitive startup funds and research space with the expectation that the successful candidate will establish, or bring to the University, a vigorous, externally funded research program.

We seek a colleague whose research will mesh with that of one or more current faculty members with interests in molecular and cell biology, developmental biology, genetics and genomics, signal transduction, and metabolism (details available at **website: http://www.bc.edu/biology).** We are particularly interested in candidates who utilize strategies such as advanced live cell imaging, systems biology, or high throughput approaches to a basic problem in cell or developmental biology. In addition to developing an active research program, the successful candidate will be expected to train graduate students, and to participate in the undergraduate teaching mission of the Department. This appointment will begin on or after July 1, 2008.

Applicants should submit curriculum vitae and a statement of present and future research plans, and arrange to have three letters of reference sent to: **Biology Search Committee, Boston College Biology Department, 140 Commonwealth Avenue, Higgins Hall, Chestnut Hill, MA 02467.**

Applications should be received by November15, 2007, to assure full consideration. Review of applications will continue until the position is filled.

Boston College is an Affirmative Action, Equal Opportunity Employer. In concert with our Jesuit, Catholic mission, Boston College is dedicated to the goal of building a culturally diverse and pluralistic faculty and staff committed to undergraduate and graduate education, and working in a multicultural environment, and strongly encourages applications from women, minorities, individuals with disabilities, and covered veterans.

UNIVERSITY of CALIFORNIA, SAN DIEGO
PH.D. Program

PLANT SYSTEMS BIOLOGY, University of California, San Diego, Salk Institute, The Scripps Research Insititute, National Science Foundation Integrative Graduate Education and Research Traineeship Graduate Training Program. This interdisciplinary training Program will train graduate students with different backgrounds at the interface of biological systems modeling, computational genomics and plant sciences and will position Ph.D. students at the frontier of systems biology. The Program will include focused mentoring of each student in two laboratories by two advisors from distinct disciplines (e.g. systems engineering/bioinformatics and plant biology). Over 30 internationally leading laboratories in diverse disciplines are participating in this new Program. For further information see **website: http://biology.ucsd.edu/psbigert.**

Highly qualified candidates with diverse backgrounds and degrees in computer sciences, engineering, biology, physics, biophysics, mathematics, chemistry, or related subjects are invited to apply by December 11, 2008. Online application submission preferably to **website: http://www.biology.ucsd.edu/grad/index.html** or alternatively applicants can apply to the University of California San Diego Bioengineering or Computer Sciences and Engineering Graduate Programs. Each application should indicate your interest in the Plant Systems Biology Program. For further information contact: **Program Directors Julian Schroeder (e-mail: jischroeder@ucsd.edu)** and **Steve Briggs (e-mail: sbriggs@ucsd.edu).**

POSITIONS OPEN

ELECTROCHEMISTRY

The Department of Chemistry at the University of Alabama is seeking an outstanding individual with expertise in electrochemistry to fill a tenure-track position at the rank of **ASSISTANT PROFESSOR.** Candidates working in all areas of electrochemistry will be considered. Specific areas of interests include, but are not limited to, biosensors, materials chemistry, and electrocatalysis. Candidates are expected to have a Ph.D. and postdoctoral training in chemistry or an allied field. The successful candidate will be dedicated to excellence in education at the undergraduate and graduate levels and be expected to develop a vigorous, externally funded research program. Further information about the Department is available at **website: http://www.bama.ua.edu/~chem.** Information about multidisciplinary research opportunities can be found at **website: http://bama.ua.edu/~chem/research/researchcenters/centers.html.** Women and members of groups underrepresented in science are especially encouraged to apply. All candidates must provide curriculum vitae including publication list, research plans (two to three pages), statement of teaching philosophy and interests (one to two pages), and arrange to have three letters of recommendation sent to: **Electrochemistry Search Committee, Department of Chemistry, The University of Alabama, P.O. Box 870336, Tuscaloosa, AL 3547.** Review of applicants will begin December 1, 2007, and continue until the position is filled. *The University of Alabama is an Equal Opportunity/Affirmative Action Employer.*

TENURE-TRACK VERTEBRATE PHYSIOLOGIST
Department of Biology, Earlham College

We seek an individual that is first and foremost excited about teaching physiology, in lecture, laboratory, and research venues, to bright and motivated undergraduates in a nationally ranked department, at a small liberal arts college. Teaching responsibilities include a human physiology course(s), an upper-level specialty course, and contributions to team-taught introductory courses in cell physiology and genetics. A commitment to collaborative student-faculty research, and an ability to bridge our departmental strengths between cellular/molecular and whole organism biology are essential. Applicants who have an interest in one or more of the following are especially attractive: comparative physiology, anatomy, systems biology, use of omics tools. Ph.D. or equivalent required; teaching or postdoctoral experience desirable. Review of applications begins November 1, 2007. Send curriculum vitae, three letters of reference, and statements describing research interests and teaching philosophy to: **Dr. Peter Blair, Department of Biology, Earlham College, Richmond, IN 47374. (Website: http://www.earlham.edu/~biol/.)** *Earlham College is an Affirmative Action/Equal Opportunity Employer. We particularly encourage applications from women, racial minorities, and Quakers.*

ASSISTANT PROFESSOR
University of Southern California
Experimental Nano-Bio Physics

The Department of Physics and Astronomy at the University of Southern California invites applications for an Assistant Professor position in the area of experimental physics at the interface of nanoscience and biological physics. The University has a strong commitment to advanced interdisciplinary research and education, and provides ample opportunities for collaborative work in the field. Applications must include detailed curriculum vitae, a statement of current and planned research directions, and the names of at least four professional references. A Ph.D. is required. Please send material to: **Werner Däppen, Chair, Department of Physics and Astronomy, University of Southern California, Los Angeles, CA 90089-0484 U.S.A.** or by **e-mail: dappen@usc.edu.** *USC values diversity and is committed to Equal Opportunity in employment. Women and men, and members of all racial and ethnic groups are encouraged to apply.*

NEUROSCIENTIST

www.aucmed.edu

The American University of the Caribbean, School of Medicine (AUC), an accredited institution with over 3500 graduated physicians, seeks to appoint a Neuroscientist.

The university seeks a Neuroscientist with a Ph.D. degree in either Neuroscience, Neuroanatomy, or Neurophysiology, or an M.D. degree with teaching experience in Neuroscience. All applicants should have teaching experience in LCME-accredited schools. Candidates with an enthusiasm for teaching medical students that have the ability to integrate neuroscience with other basic clinical sciences will be given preference.

The position is to be fulfilled at the Basic Sciences campus on the island of St. Maarten in the Netherlands Antilles, approximately 3 hours by air from Miami. AUC possesses an exceptional faculty composed of both basic scientists and clinicians. Students complete their basic sciences training on the island, and then go on to complete clinical clerkships in the U.S., U.K. or Ireland.

Interested parties should send a brief statement of teaching philosophy, their CV, and contact information for three professional references to Dr. Susan DeMesquita, Chair of the Neuroscience Search Committee, at sdemesquita@aucmed.edu.

American University of the Caribbean
School of Medicine

UNIVERSITY OF SOUTH CAROLINA

MUSC
MEDICAL UNIVERSITY OF SOUTH CAROLINA

PHARMACOLOGY/CHEMICAL BIOLOGY/MEDICINAL CHEMISTRY

ENDOWED CHAIR
SOUTH CAROLINA CENTERS OF ECONOMIC EXCELLENCE

The University of South Carolina (USC) in Columbia, SC, and the Medical University of South Carolina (MUSC) in Charleston, SC, are jointly seeking applications and nominations for an endowed chair. Individuals with demonstrated expertise in the areas of **Pharmacology, Chemical Biology,** or **Medicinal Chemistry** are encouraged to apply. The successful candidate will be an established scientist who has a strong reputation in research, has a productive record of publication and extramural funding, and is qualified for a tenured appointment at the level of Full Professor. The chair and associated laboratory spaces will be located at USC, with a joint appointment at MUSC. The chair holder will play a key role in the growth and development of research and drug discovery in the State of South Carolina. He/she will be expected to participate in professional and graduate education, and to maintain a nationally recognized, extramurally funded research program.

USC and MUSC have several Centers of Economic Excellence, including the Centers for Drug Discovery and Cancer Therapeutics, and are continuing a period of rapid growth in research. State-of-the-art core research facilities exist at both institutions, fostering a variety of collaborative research efforts and interactions.

Interested candidates should submit curriculum vitae, statements of research interests and accomplishments, and the names of three references to: **Dr. Sondra Berger, Department of Pharmaceutical and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, SC 29208** (email: **berger@cop.sc.edu**). Nominations are also welcomed. Review of applications will begin on **December 1, 2007** and will continue until the position is filled.

The University of South Carolina and the Medical University of South Carolina are Affirmative Action/Equal Opportunity Employers.

Department of Biology
University of New Mexico

The Department of Biology at the University of New Mexico invites applications for a faculty position at the **Assistant Professor** level. The appointment will be the first of two hires in the area of **Cell Biology**, and will be probationary leading to a tenure decision. The successful candidate is expected to maintain a nationally competitive, externally funded research program utilizing an established model organism. Applicants whose research focus complements existing strengths in the Department, which include developmental biology, molecular biology, genomics and molecular genetics, will be preferred. Successful candidates will be expected to participate in undergraduate and graduate teaching, and should be enthusiastic about working in a vigorous biology department with diverse research programs. Candidates must have a Ph.D. and at least two years of post-doctoral experience by the start date of the position. For complete job requirements see **http://biology.unm.edu**. To apply applicants must submit a signed letter of interest, curriculum vitae, recent reprints, statements of research and teaching interests and have at least three letters of recommendation sent to: **Cell Biology Search Committee, Department of Biology, 1 University of New Mexico, MSC03 2020, Albuquerque, NM 87131**. Review of applications will begin on **November 26, 2007**. The position will remain open until filled.

Minorities, women, veterans, and persons with disabilities are encouraged to apply. UNM is an Equal Opportunity/Affirmative Action Employer and Educator.

Tenure-Track Faculty Positions
Bio-Medical Informatics, Computational and Systems Biology, Chemical Informatics
University of California, Irvine

Two junior tenure-track positions are available at the University of California, Irvine in all areas of research at the intersection of life and computational sciences. These appointments will be made in the Donald Bren School of Information and Computer Sciences with joint appointments in the School of Biological Sciences, the School of Physical Sciences, or the School of Medicine if desirable. Exceptionally qualified senior candidates also will be considered for tenured positions. These positions will be coordinated with the interdisciplinary research programs of the UCI Institute for Genomics and Bioinformatics. For an overview of the IGB, see www.igb.uci.edu.

Examples of general areas of interest include: bioinformatics, chemoinformatics, computational biology, systems biology, synthetic biology, and medical informatics. Examples of specific areas of interest include: protein structure and function prediction; molecular simulations and docking; computational drug screening and design; comparative genomics; analysis of high-throughput data; mathematical modeling of biological systems; medical imaging. Research methods should encompass computational, statistical, or machine-learning approaches.

UCI is targeted as a growth campus for the University of California. It is one of the youngest UC campuses, yet ranked 10th among the nation's best public universities by *US News & World Report*. Salary and other compensation (including priority access to on-campus faculty housing) are competitive with the nation's finest public universities. For an overview of UCI, see **http://www.uci.edu**.

The Bren School of ICS is one of eleven academic units at UCI and was recently elevated to an independent school by the UC Regents. ICS' mission is to lead the innovation of new information and computing technology and study its economic and social significance while producing an educated workforce to further advance technology and fuel the economic engine. The Bren School of ICS has excellent faculty, innovative programs, high quality students and outstanding graduates as well as strong relationships with high tech industry. With approximately 1000 undergraduates, 100 masters and 275 doctoral students, and 70 faculty members, ICS is one of the largest computing programs in the country. The Bren School of ICS just dedicated a contemporary high-tech building designed to enhance collaborative research and education. For a perspective on ICS, see **http://www.ics.uci.edu**.

Screening will begin immediately upon receipt of a completed application. Applications will be accepted until positions are filled, although maximum consideration will be given to applications received by January 15, 2008. Completed applications containing a cover letter, curriculum vitae, sample research publications, and three to five letters of recommendation should be uploaded electronically. Please refer to the following web site for instructions: **http://www.ics.uci.edu/employment/employ_faculty.php**.

The University of California, Irvine is an Equal Opportunity Employer committed to excellence through diversity, has a National Science Foundation Advance Gender Equity Program, and is responsive to the needs of dual career couples.

POSITIONS OPEN

GENOMICS PROJECT SCIENTISTS

The Center for Genomics and Bioinformatics (**website: http://cgb.indiana.edu**) at Indiana University (Bloomington) seeks scientists (B.S./M.S./Ph.D.) for the position of **PROJECT SCIENTIST** to join the Drosphilia Genomics Resource Center and modENCODE projects in the Andrews Laboratory. Successful applicants will conduct research in the areas of genomics, molecular biology, development, genetics, and biochemistry. Duties will include research under the supervision of scientists, maintaining accurate and complete laboratory records including notebooks and electronic files, analyzing experimental data, and communicating results to scientific colleagues through oral and written reports. Appointments will be at the rank of **RESEARCH ASSOCIATE;** salary will be based on a candidate's preparation and prior experience. Direct all inquires to **e-mail: jobs@cgb.indiana.edu.** Positions are open now and applications will be accepted until positions are filled. Those received by November 26, 2007, will be assured full consideration. Please submit curriculum vitae and a description of your background interests, and have three letters of recommendation sent directly to us. Send materials to: **Position #CGB-013, Center for Genomics and Bioinformatics, Indiana University, 1001 E. 3rd Street, Bloomington, IN 47405-3700.** *Indiana University is an Affirmative Action/Equal Opportunity Employer.*

TENURE-TRACK POSITION
Plant Physiology
Connecticut College, Department of Botany

Connecticut College invites applications for an **ASSISTANT PROFESSOR** position in the Department of Botany with expertise in plant physiology. Ph.D. and evidence of teaching and research excellence required. Expertise in the physiology of higher plants in marine systems preferred. Position involves teaching courses in comparative physiology (using examples of plants and animals), plant structure and function, coastal marine biology; participation in an introductory organisms course; and development of a research program that includes undergraduates. The College Arboretum includes 450 acres of collections, natural and experimental areas, a five-acre tidal salt marsh, and access to the Thames estuary. Connecticut College is a highly selective liberal arts institution committed to interdisciplinary teaching, research, and faculty diversity. Applications should include a cover letter; curriculum vitae; copies of transcripts; statements about teaching philosophy and research interests; and three letters of reference sent directly to: **Plant Physiology Search Chair, P.O. Box 5362, Connecticut College, 270 Mohegan Avenue, New London, CT 06320.** Review of applications will begin December 3, 2007. See **website: http://www.conncoll.edu** for more information. *Affirmative Action/Equal Opportunity Employer.*

ASSOCIATE RESEARCH SCIENTIST
COLUMBIA UNIVERSITY
Neurology Department

A **TECHNICAL RESEARCH** position is available in the Neurology Department at Columbia University. Applicant will contribute to a research program investigating the molecular genetics and pathogenesis of glycogen lipid storage diseases. Basic skills in molecular and biochemical techniques required. Applicants must have a Ph.D. in molecular biology, biochemistry, or related field. Experience required in basic research involving methods of molecular biology or biochemistry. Laboratory animal experience is also required. Candidate should have strong organizational skills.

Please send curriculum vitae and addresses for three letters of recommendation by e-mail to **Dr. Salvatore DiMauro** at **e-mail: sd12@columbia.edu.**

Columbia University is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

MEMORIAL UNIVERSITY

TENURE-TRACK FACULTY POSITION in BIOINFORMATICS
Memorial University of Newfoundland

The Departments of Computer Science and Biology at Memorial University invite applications for a tenure-track position in bioinformatics starting no later than September 1, 2008. Appointment will be at the **ASSISTANT PROFESSOR** level, with primary appointment in computer science, and equal responsibility in both Departments.

A Ph.D. in computer science, computational science, biology, or related fields is required, and postdoctoral or equivalent experience is desirable. Applicants should have experience in bioinformatics, and be keen to do interdisciplinary work between the Departments. Applicants should possess a strong research record with outstanding promise for future research, and be able to demonstrate the potential for excellent undergraduate and graduate teaching in bioinformatics.

Closing date for applications will be January 5, 2008. Details and information on the application procedure may be found at **website: http://www.cs.mun.ca/ or http://www.mun.ca/biology/Home/** (reference # VPA-COSC-2007-001).

Memorial University is committed to employment equity and encourages applications from qualified women and men, visible minorities, aboriginal people and persons with disabilities. All qualified candidates are encouraged to apply; however, Canadians and permanent residents will be given priority.

TWO TENURE-TRACK POSITIONS in CELL and DEVELOPMENTAL BIOLOGY

The Department of Biology at McGill University invites applications for two positions in cell and developmental biology. Candidates using genetically well-characterized animal, plant, or fungal model systems are particularly encouraged to apply, as are applicants focusing on subcellular structures using advanced imaging and microscopy techniques or single-molecule manipulations.

The successful candidates will be joining the Developmental Biology Research Initiative (DBRI), a dynamic, interactive group of researchers working on a range of subjects in yeast, *C. elegans*, *Drosophila*, *Xenopus*, mice, and *Arabidopsis* (**website: http://www.biology.mcgill.ca/DBRI/dbri_home.htm**). The DBRI has completed a $19.8 million infrastructure renovation and renewal project, and is an integral part of the McGill University Life Sciences Research Complex. The successful candidates will be provided with ample research space in the new Bellini Life Sciences Building which will open in May 2008. We anticipate that these positions will be filled at the **Assistant Professor** (tenure track) level, but applications from more established candidates may be considered for recruitment at the **ASSOCIATE or FULL PROFESSOR** rank. Competitive startup and equipment funding packages will be available. The successful candidate is expected to contribute to undergraduate and graduate teaching in the Department and to maintain an externally funded research program.

Applicants should possess a Ph.D. degree and significant postdoctoral experience resulting in research publications. Persons wishing to be considered for these positions should forward curriculum vitae, a statement of research interests, a statement of teaching interests, copies of major publications, and arrange to have three letters of reference submitted directly to: **Cell and Developmental Biology Search, c/o Ms. Zabrina Kadkhodayan, Department of Biology, McGill University, 1205 Docteur Penfield Avenue, Montreal, Quebec, H3A 1B1, Canada.** The application deadline is 10 December 2007.

In accordance with Canadian immigration regulations, this advertisement is directed in the first instance to Canadian citizens and landed immigrants, however, all qualified candidates are encouraged to apply.

POSITIONS OPEN

SENIOR FACULTY POSITION

The Department of Biochemistry and Molecular Biology at the Louisiana State University (LSU) Health Sciences Center in New Orleans invites applications for a 12-month tenure-track appointment at the **ASSOCIATE or FULL PROFESSOR** level. This position offers generous laboratory space, a competitive professional development package and salary, and access to well-developed core facilities. The Department and Health Sciences Center are experiencing a period of unprecedented growth, with a significant increase in faculty size already underway. The LSU Health Sciences Center is located in downtown New Orleans, a vibrant historic city and commercial center of the Gulf Coast. Additional information concerning the Health Sciences Center and Department can be reviewed at **website: http://www.medschool.lsuhsc.edu/biochemistry.** The successful applicant will have a Ph.D. and/or M.D. degree and relevant postdoctoral and faculty research experience. The research interest of the faculty candidate is open to any area of biochemistry, cell, or molecular biology. In addition to a history of productive research, as evidenced by high quality publications, the successful candidate is expected to have ongoing extramural funding. Modest teaching responsibilities will involve participation in service courses (medical, dental) and/or teaching at the graduate level. Electronic or paper applications are welcome. Include curriculum vitae, statement of present and future research interests, and the names and addresses of at least three references. Electronic submission should be e-mailed to **e-mail: wvedec@lsuhsc.edu.** Paper submissions should be sent to:

Wayne V. Vedeckis, Ph.D.
Search Committee Chairman
Department of Biochemistry and Molecular Biology
Louisiana State University Health Sciences Center
1901 Perdido Street
New Orleans, LA 70112

LSUHSC is an Equal Opportunity/Affirmative Action Employer.

TENURE-TRACK MICROBIOLOGIST

The Department of Biology at the University of Minnesota, Duluth (UMD) invites applications for a tenure-track **ASSISTANT PROFESSOR** position in microbiology beginning August 2008. We seek outstanding candidates who use molecular and complementary approaches to investigate microorganisms. Laboratory space will be provided in the new state-of-the-art Swenson Science Building along with a competitive startup package. Opportunities exist for collaborations with researchers at the Natural Resources Research Institute, School of Medicine, College of Pharmacy, Large Lakes Observatory, and the EPA Mid-Continent Ecology Division. Teaching responsibilities include general microbiology, either a microbial physiology, immunology, or virology course, and an advanced course in the applicant's research specialty. We desire applicants who are committed to excellence in teaching, the development of an innovative, externally funded research program, and training of graduate and undergraduate research students. Candidates must have a Ph.D. in the biological sciences, potential for excellence in teaching and research, and communication skills to support quality teaching. Only online applications will be accepted. Please initiate the online application at **website: https://employment.umn.edu/applicants/jsp/shared/frameset/Frameset.jsp?time=1191968351321** and search postings for job **#151304.** In addition, please have three letters of reference sent to: **Chairperson, Microbiologist Search Committee, Department of Biology, SSB 207, 1035 Kirby Drive, University of Minnesota Duluth, Duluth, MN 55812.** Review of complete applications will begin November 12, 2007, and continue until the position is filled. Visit our Department at **website: http://www.d.umn.edu/biology.** Abundant recreational opportunities and a high quality of life complement the thriving intellectual and artistic atmosphere in the region. *The University of Minnesota is an Equal Opportunity Educator and Employer.*

University of California, Berkeley

ASSISTANT PROFESSOR IN RESTORATION ECOLOGY

The Department of Environmental Science, Policy and Management (ESPM) at the University of California, Berkeley (**http://espm.berkeley.edu**) invites applications for a tenure-track, nine-month (academic year) faculty position in Restoration Ecology available starting July 1, 2008. The position includes a joint appointment in the California Agricultural Experiment Station. There are important interdisciplinary connections to the Department of Integrative Biology, and Landscape Architecture and Environmental Planning.

Applicants should possess a Ph.D. in ecology or a closely related discipline. Candidates may conduct research on a variety of contemporary and interdisciplinary problems relating to restoring habitats to promote ecosystem functions or services, and biodiversity from local to landscape scales. We expect their research to contribute to scholarly and applied progress in restoration ecology based on quantitative ecological understanding including, but not limited to, areas such as community assembly processes, ecological interactions, and ecosystem dynamics, and the integration of ecological and socio-economic disciplines. The candidate will be expected to teach an upper division course on Restoration Ecology and a graduate seminar annually, and periodically teach in environmental science courses.

We particularly encourage applications from women and under-represented ethnic minorities. Electronic submissions are preferred as a single PDF file and emailed to **restorationecology@nature.berkeley.edu**. An application should include a curriculum vitae, statements of research and teaching interests, and recent publications.

Three letters of recommendation should be mailed separately to:

Ms. Vinaya Gokarn, Chair's Assistant
Restoration Ecology Search Committee
ESPM, 137 Mulford Hall #3114
University of California
Berkeley, CA 94720-3114

Refer potential reviewers to the UC Berkeley Statement of Confidentiality found at **http://apo.chance.berkeley.edu/evalltr.html**. Applications must be *postmarked* by **December 1, 2007**.

ASSISTANT PROFESSOR IN FOREST ECOSYSTEM MANAGEMENT

The Ecosystem Sciences Division of the Department of Environmental Science, Policy and Management (ESPM) at the University of California, Berkeley (**http://espm.berkeley.edu**) invites applications for a tenure-track, nine-month (academic year) faculty position in Forest Ecosystem Management available starting July 1, 2008. The position includes a joint appointment in the California Agricultural Experiment Station. There are important interdisciplinary connections to the Department of Integrative Biology, Agricultural and Resource Economics, Landscape Architecture, Civil and Environmental Engineering, Economics, and the Haas School of Business.

Applicants should possess a Ph.D. in forest science, natural resource management, systems ecology and modeling, environmental engineering, hydrology, or a closely related discipline. Candidates may conduct research on a variety of contemporary and interdisciplinary problems relating to forest ecosystem management and planning. We expect their research to contribute to scholarly and applied progress in forest ecosystem management based on a rigorous quantitative ecological understanding of forest dynamics, geo-spatial analyses (GIS), landscape ecology/management, global change science, natural resource planning, economics, or other quantitative areas. The candidate will be expected to teach an upper division course on Forest Ecosystem Management and a graduate seminar annually, and occasionally participate in environmental science courses.

We particularly encourage applications from women and under-represented ethnic minorities. Electronic submissions are preferred as a single PDF file and emailed to **forestmanagement@nature.berkeley.edu**. An application should include a curriculum vitae, statements of research and teaching interests, and recent publications.

Applications can also be mailed to:

Ms. Vinaya Gokarn, Chair's Assistant
Forest Ecosystem Management Search Committee
ESPM, 137 Mulford Hall #3114
University of California
Berkeley, CA 94720-3114

Please also arrange three letters of recommendation to be mailed separately to the above address.

Refer potential reviewers to the UC Berkeley Statement of Confidentiality found at **http://apo.chance.berkeley.edu/evalltr.html**. Applications must be *postmarked* by **December 1, 2007**.

The University of California at Berkeley is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

ASSISTANT PROFESSOR TWO POSITIONS in STEM CELL SCIENCE or STEM CELL MEDICINE Biology, Medicine, Engineering, Physical Sciences, or Any Other Pertinent Field

The University of California, San Diego (UCSD) Stem Cell Program at the University of California, San Diego is seeking to recruit two or more tenure-track Assistant Professors who conduct strongly interdisciplinary research in stem cell science or stem cell medicine. These two tenure-track faculty positions are available in fall 2008 at the Assistant Professor level; appointments at other ranks will be considered based on qualifications and availability of funds.

The Program seeks candidates who will establish independent and vigorous extramurally funded research programs in stem cell biology, biochemistry, medicine, engineering, or other fields related to stem cell science, with innovative approaches and expertise in more than one discipline. Candidates should have a track record of publications in internationally recognized journals, and a willingness to participate in graduate and undergraduate teaching. Applicants must possess a Ph.D. or M.D. degree.

The UCSD Stem Cell Program is an interdisciplinary and collaborative research and teaching program focused on using stem cells to understand basic biology and the causes and treatment of human disease. The Program seeks to improve human health by fostering innovation and collaboration and by providing mentoring and frequent opportunities for cross-disciplinary interaction among faculty. The successful applicants will have appointments in one or more home departments on the UCSD general campus or health sciences.

You may also send your curriculum vitae, a statement of research experience and interests, and the names and e-mail addresses of three references to **e-mail: ibraswell@ucsd.ecu.**

For more information on our Program, and to apply, please go to **website: http://stemcells.ucsd.edu/.**

Review of applications will begin November 1, 2007, and the search will continue until positions are filled.

Equal Opportunity/Affirmative Action Employer.

FACULTY POSITION, GENETICS, GENOMICS, or EPIGENETICS. The Department of Biochemistry and Molecular Biology at the Penn State University College of Medicine invites applications for a full-time, tenure-track position. We seek candidates with already established highly competitive research programs in the areas of molecular genetics, epigenetics, and/or genomics. For additional information, please visit the following **website: http://www.hmc.psu.edu/biochemistry/.** Interested applicants should submit curriculum vitae, a brief statement of research plans, and arrange to have three letters of reference sent to: **Judith S. Bond, Ph.D., Professor and Chair, Department of Biochemistry and Molecular Biology H171S, P.O. Box 850, Penn State University College of Medicine, Hershey, PA 17033.** *Penn State is committed to Affirmative Action, Equal Opportunity and diversity.*

POSTDOCTORAL POSITION in THEORETICAL ECOLOGY

A Postdoctoral Fellowship is available for a National Science Foundation-funded project on consumer-resource dynamics. The objective is to develop theory on multiple coexistence mechanisms in communities with competition and predation. The appointment is for two years starting in January 2008. Applicants should have a Ph.D. in mathematical biology, theoretical ecology, applied mathematics, or some related discipline, and have skills in both analytical and numerical approaches to population dynamics. Applicants should submit curriculum vitae, a statement of research interests and accomplishments, and arrange to have three letters of reference sent to **Dr. Priyanga Amarasekare (e-mail: amarasek@eeb.ucla.edu).** *The University of California is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

PENNSTATE

FACULTY POSITION in the BASIC LIFE SCIENCES

The Department of Biochemistry and Molecular Biology at the Pennsylvania State University invites applications for a tenure-track position at the **ASSISTANT PROFESSOR** level in all areas of biochemistry, molecular biology, microbiology, virology, cell and developmental biology, systems biology, and genomics. The Department, located on the University Park campus in State College, Pennsylvania, is in the Eberly College of Science and has 40 faculty members whose research explores a wide range of biological questions at the molecular level. Our faculty collaborate extensively with other life science researchers across Penn State and have access to state-of-the-art research facilities through the Huck Institutes of the Life Sciences. Additional information about the Department may be found at **website: http://www.bmb.psu.edu.**

The position is available for fall 2008. Review of applications will begin December 1, 2007, and will continue until the position is filled. Applications should include curriculum vitae and statements of research and teaching interests in a single electronic PDF file sent to **e-mail: njw12@psu.edu.** Three letters of reference should be sent to the same e-mail address.

Penn State is committed to Affirmative Action, Equal Opportunity and the diversity of its workforce.

Adelphi University, Garden City, New York, invites applications for a tenure-track **ASSISTANT PROFESSOR of BIOLOGY-GENETICS** position in any area of genetics to begin August 2008. Ph.D required; postdoctoral experience preferred. Excellent potential as a teacher, significant research accomplishments, and the potential to develop a fundable independent research program involving undergraduates and Master's students are required. Teaching responsibilities will include undergraduate genetics with laboratory and could also include introductory biology, upper-level undergraduate, or graduate courses. Those who combine the ability to teach genetics with expertise in ecology, evolution, botany, biotechnology, genomics, or microscopy (including electron microscopy) are especially encouraged to apply, although other specialties will be considered. For more information about the Department, visit **website: http://academics.adelphi.edu/artsci/bio/.** Adelphi is a private university with the spirit of a liberal arts college, committed to combining teaching and scholarship, and located in suburban Long Island within easy reach of New York City. Deadline for applications: December 10, 2007. Please apply at **website: http://www.adelphi.edu/positions/faculty.** All appointments are subject to final approval by the Board of Trustees. For additional information about Adelphi University please visit our **website: http://www.adelphi.edu.** *Adelphi University is committed to building a diverse faculty and strongly encourages applications from minority and women candidates. Adelphi University is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION Washington University School of Medicine Pain Center

A Postdoctoral position available at Washington University School of Medicine Pain Center to study the molecular mechanisms of itch sensation using mouse as an animal model. Candidates should have a Ph.D. degree in neurobiology or molecular biology. Please send curriculum vitae to **e-mail: chenz@wustl.edu,** or to: **Zhou-Feng Chen, Department of Anesthesiology, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110.** The Laboratory **website: http://elysium.wustl.edu/zclab/.**

POSITIONS OPEN

CURATOR of MARINE INVERTEBRATE SYSTEMATIST

The American Museum of Natural History (AMNH) is seeking a scientist to fill a tenure-track position in the field of invertebrate systematics, with emphasis on living marine taxa, beginning July 2008. The successful candidate will be appointed at the rank of **ASSISTANT CURATOR** with salary commensurate with experience and accomplishment. Candidates will be expected to develop an externally funded research program in systematics and/or phylogenetics of invertebrates including field work and the use of diverse character systems (such as development) and ability to oversee relevant segments of museum collections and staff. Areas of taxon research focus can include any metazoan marine invertebrate group. Applicants must have a strong commitment to the use and continued development of museum collections as a complement to their research programs.

Application materials should include: curriculum vitae, statement of research interests and goals, teaching statement for graduate instruction in the AMNH Richard Gilder Graduate School, and copies of publications relevant to the application, and names and contact information of at least three individuals able to comment on the capabilities of the candidate. The deadline for submission of application materials is December 14, 2007. Interviews will be conducted by early 2008, in conformance with AMNH recruitment and conflict of interest policies. Materials should be sent to: **Chair, Marine Invertebrate Search, Division of Invertebrate Zoology, The American Museum of Natural History, New York, NY 10024.** Alternatively application materials may be directed to **e-mail: egaughan@amnh.org.**

Closing date for submission of materials is December 14, 2007.

Electronic submissions encouraged and preferred. *Equal Employment Opportunity.*

ALTERNATIVE ENERGY CHEMISTRY POSITION

The Department of Chemistry at the University of Alabama seeks an outstanding individual with expertise in chemistry-related alternative energy to fill a tenure-track position at the **ASSISTANT PROFESSOR** rank. Candidates working in all areas of chemistry with multidisciplinary interests and whose work is focused on the central theme of alternative energy will be considered. We are especially interested in candidates taking a multifaceted approach in terms of tools and approaches. Successful candidates are expected to have a Ph.D. and postdoctoral training in chemistry or closely allied field and to develop a vigorous, externally funded research program. Commitment to excellence in both undergraduate and graduate teaching is also required. Further information on the Department is available at **website: http://www.bama.ua.edu/~chem/.** All candidates should provide curriculum vitae including publication list, research plans (three to five pages), teaching plans (two pages), and arrange to have three letters of recommendation sent to the: **Alternative Energy Chemistry Search Committee, Department of Chemistry, The University of Alabama, P.O. Box 870336, Tuscaloosa, AL 35487.** Review of applicants will begin December 1, 2007, and continue until the position is filled. *The University of Alabama is an Equal Opportunity/Affirmative Action Employer. Women and members of groups underrepresented in science are especially encouraged to apply.*

POSTDOCTORAL POSITIONS available in a multidisciplinary group to study protein structures at the Fox Chase Cancer Center in historic Philadelphia. The main focus of these positions will be the study of the structure and dynamics of scaffolding proteins in ion channel or cell receptor assemblies. Experiences with one or more of the following areas will be considered: molecular biophysics, protein biochemistry, membrane biochemistry/biophysics, cell biology/biophysics, nuclear magnetic resonance, X-ray and neutron scattering, electrophysiology, or fluorescence microscopy.

To apply, send curriculum vitae and names of references by e-mail attachment to **e-mail: zimei.bu@fccc.edu.**

POSITIONS OPEN

ASSISTANT PROFESSOR, ENTOMOLOGY
Medical - Veterinary Entomologist
Oklahoma State University

The Department of Entomology and Plant Pathology, Oklahoma State University invites applications for an 11-month tenure-track faculty position in medical-veterinary entomology at the rank of Assistant Professor and with a work assignment of 70 percent research and 30 percent teaching. Applications are encouraged from individuals with training/interests in arthropods that affect the health of human and other animal populations. Preference will be given to candidates who have research experience with blood-feeding arthropods and whose research complements ongoing research programs at Oklahoma State University. The successful candidate will be expected to develop an externally funded research program, to work with multidisciplinary teams directed toward management of arthropods of public health significance and/or affect livestock production. Teaching responsibilities may include four credit hours of courses each fall and spring semester including but not limited to medical-veterinary entomology. The successful candidate will assist the undergraduate teaching coordinator with recruiting, advising and curriculum development. Graduate teaching responsibilities will include recruiting and advisement of graduate students as part of the research program. Applicants must have an earned Doctorate in entomology or a closely related field. Postdoctoral experience in medical-veterinary entomology research and teaching is preferred. Applications should include the following: (1) letter of application stating reasons for interest in this position and qualifications, (2) curriculum vitae, (3) statement of research and teaching interests, (4) official university transcripts noting date of terminal (Ph.D.) degree, and (5) the letters from four references. This information should be sent to: **Ms. Diana Ward, Department of Entomology and Plant Pathology, 127 Noble Research Center, Oklahoma State University, Stillwater, OK 74078; telephone: 405-744-9405; fax: 405-744-6039; e-mail: diana.ward@okstate.edu.** Questions regarding the position may be directed to the **Search Committee Chair, Dr. Jack Dillwith, (e-mail: jack.dillwith@okstate.edu).** Review of applications may begin November 15, 2007, and applications will be accepted until a candidate has accepted the position.

FACULTY POSITION in TOXICOLOGY/CLINICAL CHEMISTRY

Applications are invited for a position in the Department of Chemistry and Biochemistry at Florida International University (FIU) in the area of toxicology, forensic toxicology, or clinical chemistry, with an appointment starting in fall 2008. A Ph.D. and postdoctoral experience are required. Candidates are expected to develop a vigorous and externally funded research program. FIU is a public research extensive university with over 38,000 students located in west-suburban Miami, with a new medical school scheduled to open in 2009. The rapidly growing Department houses 29 faculty and 85 graduate students. Please see **website: http://www.fiu.edu/orgs/chemistry** for more details. Send curriculum vitae, transcripts, research plans, and three letters of reference to: **Toxicology Search Committee, Department of Chemistry and Biochemistry, Florida International University, Miami, FL 33199.** The selection process will begin on December 31, 2007. *FIU is an Equal Opportunity and Affirmative Action Employer.*

POSTDOCTORAL POSITIONS available immediately to study the genetics and cell and molecular biology of aging in yeast (e.g. see *Aging Cell* **6:** 405, 2007). Send curriculum vitae and three references to: **S. Michal Jazwinski, Ph.D., Tulane Center for Aging, Tulane University Health Sciences Center, 1430 Tulane Avenue, New Orleans, LA 70112.** Electronic applications **(e-mail: sjazwins@tulane.edu)** will receive prompt attention. *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

CHEMICAL BIOLOGY and BIOMEDICAL SCIENCES
McMaster University

The Departments of Chemistry and Biochemistry and Biomedical Sciences at McMaster University are seeking individuals working at the interface of chemistry and biology. Areas of particular interest include natural products, synthetic and medicinal chemistry, and bio-analytical chemistry. Appointment will be at the level of **ASSISTANT PROFESSOR,** however candidates at other levels will be considered.

This position is linked to existing strengths in the areas of chemical biology and infectious disease and to a recent major infrastructure expansion involving the two Departments. This expansion includes the construction of a new 12,000 square-foot open research facility that is equipped with state-of-the-art chromatographic, nuclear magnetic resonance and mass spectrometry instrumentation for the study of small molecule-biomolecule interactions, natural product isolation, cell growth, high-throughput, small molecule screening and synthetic chemistry.

Applicants should provide curriculum vitae, a research plan, and a statement of teaching interests and experience, and should arrange for three letters of recommendation to be sent to: **Dr. Gerry Wright Chair, Search Committee, Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street W., Hamilton, ON, L8N 3Z5.**

Applications will be considered starting October 25, 2007, and will continue until the position is filled.

In accordance with Canadian immigration requirements, Canadian citizens and permanent residents will be considered first for these positions. McMaster University is committed to employment equity and encourages applications from all qualified candidates, including members of visible minorities, aboriginal peoples, persons with disabilities, and women.

FACULTY POSITIONS in BIOLOGY
The University of Washington

The University of Washington's Department of Biology has two open tenure-track faculty positions. We welcome applicants in both core and interdisciplinary areas of biology but have particular interest in areas of cellular, molecular, and physiological levels of organization in plants or animals. A record of outstanding achievement, a promising research program, and a commitment to teaching are more important than the specific research area. Our consolidation of Botany, Zoology and Undergraduate Biology Programs into a single unit expands opportunities for new projects and interdisciplinary initiatives. Information about the Department is available at **website: http://www.biology.washington.edu.**

Appointments at the **ASSISTANT PROFESSOR** rank are anticipated. Appointments at the **ASSOCIATE or FULL PROFESSOR** rank may be considered for candidates who have demonstrated a commitment to mentoring underrepresented students in the sciences. Applicants must have earned a Doctorate by the date of appointment.

Please apply online at **website: http://www.biology.washington.edu/fachires/** and submit a cover letter, curriculum vitae, sample reprints, statements of research and of teaching interests, and names of at least three references. Applications received by November 1, 2007, will be given priority.

University of Washington faculty engage in teaching, research, and service. The University of Washington, a recipient of the 2006 Alfred P. Sloan award for Faculty Career Flexibility, is committed to supporting the work-life balance of its faculty. *The University is building a culturally diverse faculty and staff and strongly encourages applications from women, minorities, individuals with disabilities, and covered veterans. The University of Washington is an Affirmative Action, Equal Opportunity Employer.*

POSITIONS OPEN

EVOLUTION/POPULATION GENETICS

The Department of Biological Sciences at the University of Wisconsin, Milwaukee, seeks a tenure-track **ASSISTANT PROFESSOR** who is committed to excellence in undergraduate and graduate teaching, scholarly research, and service. Appointment begins August 2008. Ph.D. required by start of appointment. Candidate should have broad interests in molecular and evolutionary ecology of animals. Preference will be given to individuals doing research that incorporates an integrative approach across biological subdisciplines. The successful candidate will be expected to develop an externally funded research program and contribute to teaching in both core biology courses and an upper level population genetics course. Applicants should send a single PDF file containing a cover letter, curriculum vitae, and statements of research and teaching goals to **Craig Sandgren, Department Chair (e-mail: sandgren@uwm.edu).** In addition, three letters of reference should be sent to: **Evolution/Genetics Search, Department of Biological Sciences, University of Wisconsin-Milwaukee, P.O. Box 413, Milwaukee, WI 53201.** Screening of candidates will begin December 21, 2007, and continue until the position is filled. For more information, please see **website: http://www.uwm.edu/letsci/jobs/index.html.** *UWM is an Equal Opportunity/Affirmative Action Employer.*

HEAD of DIVERSITY
Keystone Symposia

Keystone Symposia, a not-for-profit meeting organization located in beautiful Summit County, Colorado, seeks a Head of Diversity to implement ambitious plans for enhancing diversity at its life science meetings. The ideal candidate will have excellent oral and written communications skills, be willing to travel, and be self-motivated and able to work effectively with a team. The candidate will have to interact confidently with scientists, science administrators, and potential donors. A doctoral degree in life science or medicine is highly preferred; we will consider highly qualified applicants at the Bachelor's or Master's degree level with at least three years of substantial experience in diversity. Additional desirable qualities include at least two years of postdoctoral experience in life science research, and demonstrated experience with enhancing diversity, teambuilding networking, and project management.

Send resume and cover letter to: **Human Resources Manager, Keystone Symposia, P.O. Box 1630, Silverthone, CO 80498** or **e-mail: maryjor@keystonesymposia.org.** For more information about Keystone Symposia or to view a more detailed job description you can visit our **website: http://www.keystonesymposia.org.**

The University of Kansas School of Engineering invites applications for the position of **ASSISTANT SCIENTIST for the BIOENGINEERING RESEARCH CENTER.** The Scientist will be responsible for ensuring smooth implementation of the Center's collaborative research and training Programs. The Scientist will participate in the technological research and development efforts of the Center. These responsibilities include but are not limited to active participation as a leader who writes and contributes to manuscripts, leading and participating in extramural and intramural grant applications, research presentations, and graduate laboratory instruction. The requirements for this position include a Ph.D. degree in materials science, materials engineering, material chemical science, or a related field. Qualified applicants must have a strong background in maintaining, operating, and providing training on instrumentation used for materials characterization and functional imaging of biologic constructs and material/tissue interfaces. A detailed position description with complete requirements and application procedures can be found at the mechanical engineering **(website: http://www.engr.ku.edu/me)** website.

Review of applications begins December 1, 2007, and will continue until the position is filled.

The University of Kansas is an Equal Opportunity/Affirmative Action Employer.

POSITIONS OPEN

ASSOCIATE PROFESSOR/PROFESSOR
Neuropharmacology

We seek an established investigator with outstanding research accomplishments in neuropharmacology and neuroscience to complement and enhance the diversity of basic and clinical neuroscience research strengths in our Department and Institution. Medical College of Georgia (MCG) has an eminent, well-funded neuroscience research community across the Departments of Pharmacology and Toxicology, Physiology, Cell Biology, Molecular Medicine, Psychiatry and Health Behavior, and Neurology. Faculty members in the Department of Pharmacology and Toxicology conduct research that is relevant to a range of neurological and psychiatric disorders including Alzheimer's disease, Parkinson's disease, drug abuse, schizophrenia, and mental retardation. We seek applicants with an established record of productivity and an extramurally funded research program, particularly a program that can be translated to clinical problems. Applicants with expertise in drug discovery and development, cell signaling, systems neurobiology, or neurotoxicology are particularly encouraged to apply. Strong consideration will be given to applicants with a clear collaborative potential with current faculty members in the Department of Pharmacology and Toxicology. We offer a generous startup package, excellent laboratory space and outstanding core facilities for microarray technology, genetically modified animals, cell imaging, electron microscopy, small animal and nonhuman primate behavior, and clinical collaborations. The successful applicant will participate in teaching programs for professional and graduate students. Please send curriculum vitae, summary of professional and research goals, and the names and addresses of three references to: **Search Committee, c/o Dr. Alvin V. Terry, Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300, e-mail: aterry@mail.mcg.edu,** and visit the Department homepage **(website: http://www.mcg.edu/SOM/phmtox/index.html).** Application review will begin September 17, 2007. Deadline for applications is November 1, 2007. *MCG is an Equal Employment Opportunity/Affirmative Action/Equal Access Employer.*

HARVARD UNIVERSITY and The CHILDREN'S HOSPITAL BOSTON

The Stem Cell Program at Children's Hospital Boston invites applications for an **ASSISTANT PROFESSOR** position (tenure track). This position will be a joint appointment between Harvard University's newly established Department of Stem Cell and Regenerative Biology and the Stem Cell Program at Children's Hospital Boston. Both are affiliated with the Harvard Stem Cell Institute. Candidates must hold a Ph.D. and/or M.D. Outstanding scientists with a demonstrated research interest in stem cells and regenerative biology/medicine will be given preference. This could include chromatin or transcriptional regulation, chemical biology, tissue regeneration, cancer or disease models.

Applicants must submit an electronic copy of current curriculum vitae and a description of current and proposed research plans to: **Leonard I. Zon, M.D., Director, Stem Cell Program at Children's Hospital Boston** (e-mail these materials to **e-mail: ckent@enders.tch.harvard.edu**) and should arrange to have three letters of recommendation mailed directly from the references to: **Search Committee, Leonard I. Zon, M.D., Stem Cell Program at Children's Hospital Boston, 300 Longwood Avenue/Karp 08.215, Boston, MA 02115.** Application review will begin on October 15, 2007, and will continue until the position is filled. *Children's Hospital Boston and Harvard University are Equal Employment Opportunity Employers.*

POSITIONS OPEN

U.S. POSTAL SERVICE

Statement required by the Act of 12 August 1970, Section 3685, Title 39, United States Code, showing the ownership, management, and circulation of:

1-9. *Science*, Publication No. 0036-8075, is published weekly on Friday, except the last week in December, at 1200 New York Avenue, N.W., Washington, DC 20005. Date of filing: 26 September 2007. This is also the address of the publisher, the editor, and the managing editor, who are, respectively, Beth Rosner, Donald Kennedy, and Monica M. Bradford.

10. The owner is the American Association for the Advancement of Science, 1200 New York Avenue, N.W., Washington, DC 20005. Stockholders: None.

11. Known bondholders, mortgages, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages, or other securities: None

12. The purpose, function, and nonprofit status of this organization and the exempt status for federal income tax purposes have not changed during the preceding 12 months.

13-15. The average number of copies of each issue during the preceding 12 months is (A) Total number of copies printed: 129,756; (B) Paid circulation: 121,515; (1) Paid/Requested outside-county mail subscriptions stated on form 3541: 100,434; (2) Paid/Requested in-county subscriptions stated on form 3541: 0; (3) Sales through dealers and carriers, street vendors, counter sales: 21,046; (4) Other classes mailed through USPS: 35; (C) Total paid circulation: 121,515; (D) Free distribution: samples, complimentary, and other free copies: 7,195; (1) Outside-county as stated on form 3541: 2,394; (2) In-county as stated on form 3541: 0; (3) Other classes mailed through the USPS: 4; (E) Free distribution outside of mail: Carrier or other means: 4,797; (F) Total free distribution: 7,195; (G) Total distribution: 128,710; (H) Copies not distributed: 1,046; (I) Total: 129,756; (J) Percent paid and/or Requested Circulation: 94.4%.

Actual number of copies of single issue (9/14/2007) published nearest to filing date are (A) Total number of copies printed: 126,300; (B) Paid circulation: 118,293; (1) Paid/Requested outside-county mail subscriptions stated on form 3541: 98,350; (2) Paid/Requested in-county subscriptions stated on form 3541: 0; (3) Sales through dealers and carriers, street vendors, counter sales: 19,919; (4) Other classes mailed through USPS: 24; (C) Total paid circulation: 118,293; (D) Free distribution: Samples, complimentary, and other free copies: 6,985; (1) Outside-county as stated on form 3541: 2,400; (2) In-county as stated on form 3541: 0; (3) Other classes mailed through the USPS: 4; (E) Free distribution outside of mail: Carrier or other means: 4,581; (F) Total free distribution: 6,985; (G) Total distribution: 125,278; (H) Copies not distributed: 1,022; (I) Total: 126,300; (J) Percent paid and/or Requested Circulation: 94.4%.

I certify that the statements made above are correct and complete. (signed) Beth Rosner, Publisher.

OPPORTUNITIES for Ph.D. STUDENTS in MARINE CONSERVATION

The Center for Marine Conservation of Duke University's Nicholas School of the Environment and Earth Sciences seeks up to five Ph.D. students interested in interdisciplinary approaches to marine conservation. Qualified students will possess an undergraduate or Master's degree in an appropriate field of natural or social science, prior research experience, and strong quantitative skills. Successful candidates will work on issues in marine conservation that integrate natural and social sciences, and must work with more than one faculty mentor. A list of participating faculty is available at **website: http://www.nicholas.duke.edu/msc/faculty.php** and information on application procedures is at **website: http://www.nicholas.duke.edu/programs/doctoral/.** The application deadline to the Duke Graduate School is December 15, 2007. Interested students must contact prospective faculty members prior to submitting an application. For more information please **e-mail: marineconservation@duke.edu.**

POSITIONS OPEN

POSTDOCTORAL POSITIONS in BIOINFORMATICS available at the Gerstein Laboratory at Yale, focusing on various topics in genomics, networks, data mining, and structural analysis. See **website: http://gersteinlab.org/jobs** for more information.

POSTDOCTORAL POSITION

Two Postdoctoral positions funded by the National Institutes of Health are available, to study the roles of insulin, nitric oxide and protein tyrosine phosphatases in regulation of vascular smooth muscle cell signaling and neointima formation in vascular injury. Our projects address important basic science questions and also have relevance to clinical problems. Experience in molecular biology or rat surgery and a good command of the English language are essential. Competitive salaries are offered. Please send curriculum vitae and the names of three references to: **Dr. Aviv Hassid, Department of Physiology, University of Tennessee, 894 Union Avenue, Memphis, TN 38163. E-mail: ahassid@tennessee.edu; fax: 901-448-7126.** *The University of Tennessee is an Equal Employment Opportunity/Affirmative Action Title VI/Title IX/Section 504/ADA/ADEA Institution in the provision of its education and employment programs and services.*

ASSISTANT PROFESSOR of BIOCHEMISTRY
University of Washington

The Department of Biochemistry at the University of Washington School of Medicine invites applications for a tenure-track position as Assistant Professor. We seek creative scientists who use contemporary approaches to solve fundamental biological or biomedical problems at the molecular level. Our Department is also strongly committed to undergraduate, graduate, and medical school teaching. For additional information, visit **website: http://depts.washington.edu/biowww/.** Submit curriculum vitae, research prospectus, reprints and preprints, and three letters of recommendation (to be forwarded separately) by December 15, 2007, to: **Alan Weiner, Chair, Department of Biochemistry, University of Washington, Seattle, WA 98195-7350. Telephone: 206-543-1768.** *The University of Washington is an Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL FELLOW POSITION, University of Alabama at Birmingham, to study proteomics in kidney disease (*J. Biol. Chem.* **281:** 39681-39692, 2006; *J. Clin. Investig.* **112:**209-221, 2003). Ph.D. in biochemistry and minimum of two years of postdoctoral experience are required. Experience in proteomics or kidney disease are desirable but not necessary. E-mail curriculum vitae and summary of research experience to **Sumant S. Chugh, M.D.,** at **e-mail: chugh@uab.edu.**

MARKETPLACE